COLONIC MOTILITY IN HEALTH AND IN SLOW TRANSIT CONSTIPATION

by

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A thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

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June 2015

ABSTRACT

Introduction

Our knowledge of normal human colonic motility remains incomplete. Historically, this has been due to the relative inaccessibility of this organ for study, and the lack of standardisation of methods used to investigate it. Recent device development has provided us with advanced tools by which to assess colonic motility, namely pancolonic manometry, and the wireless motility capsule (WMC).

Using traditional diagnostic tests, a subgroup of patients presenting with severe intractable symptoms, but without organic disease, are found to have slow transit constipation (STC). This is believed to be primarily due to colonic dysmotility, although colonic motor functions remain poorly understood in this group also.

Aims

The principal aims of this thesis were to:

(1) explore the effect of pancolonic manometric recording technique on colonic motility;
(2) describe pancolonic motility in STC, compared to healthy control subjects;
(3) using the wireless motility capsule (WMC), validate the precise location of the pH fall around the ileo-caecal junction as a landmark for measuring colonic motility;
(4) obtain normative data for colonic motility (transit and contractility) and intraluminal pH in a large cohort of healthy volunteers using the WMC, and compare this to patients with STC.

Methods

The following methods were used:

(1) prolonged pancolonic manometry in healthy volunteers and patients with STC;
(2) a dual scintigraphic technique, involving radioactive-labelling of the WMC in healthy volunteers;
(3) wireless motility capsule studies of colonic motility in healthy volunteers and in patients with STC.

Results

Colonic manometric recording technique (bowel preparation or not, and different catheter types) significantly influences some characteristics of propagating sequence

(PS) activity, including frequency, amplitude, polarity, relationship between consecutive PSs, and circadian rhythm.

Patients with STC display dysregulated colonic motor function represented by disorganised spatiotemporal patterning and loss of 'regional linkage' among PSs.

The fall in pH measured by the WMC was confirmed to be either in the caecum, ascending colon, or as the capsule moved from the caecum to the ascending colon.

Using the WMC, the upper limit of normal colonic transit time (CTT) was found to be 51 h; however, CTT is not a continuous variable and exhibits peaks every 24 h. CTT is significantly prolonged in females and affected by the study protocol employed. In patients with STC, colonic contractility (motility index) is increased in comparison with healthy controls, and intraluminal pH is more acidic in the proximal colon, and more alkaline in the distal colon.

Conclusions

The method of pancolonic manometry requires standardisation. However, novel metrics derived from prolonged pancolonic recordings have improved our understanding of the physiology of colonic motor function in health, and also pathophysiology in constipation. The WMC provides an alternative, less invasive method to investigate colonic motility; this technique also requires standardisation, but early results in patients with STC complement those from manometry, and also reveal alterations in intraluminal pH that may be of pathophysiological significance.

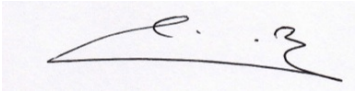
STATEMENT OF ORIGINALITY

I, Sahar D Mohammed wish to confirm that all the work presented in this thesis is original in concept, design and execution, although some of the applied techniques have been described previously, or are used in clinical practice. The author performed all of the experiments, data acquisition, analysis of the resulting data and preparation of this thesis, unless clearly stated otherwise.

In particular, some pancolonic manometric studies in healthy controls and patients with slow transit constipation (Chapters 3 and 4) were performed in collaboration with Dr Phil Dinning's group in Australia and were not repeated by the author. Dr Dinning also created colour-contoured spatiotemporal maps for colonic propagating contractions. In addition, colonoscopic-assisted placement of colonic manometric catheters (Chapters 2, 4, and 5), though observed by the author, was performed at the Royal London Hospital by both Dr Peter Fairclough and Dr Sean Preston.

For the wireless motility capsule studies (Chapter 6), Dr Mark Scott performed the radioactive labelling of the capsule. Dr Etsuro Yazaki performed the nasoileal intubation. The author observed both of these procedures. Miss Emma O'Shaughnessy performed some of the data analysis of scintigraphic recordings.

The author was additionally responsible for acquiring appropriate ethical approvals, and also recruitment of volunteers and patients.



June 2015

ACKNOWLEDGEMENTS

Since I did my MSc in gastroenterology science and my interest in the research field of colonic motility grew up. However, I am certain have not proceed further in this field without the full support and encouragement along the way from my supervisors Dr Mark Scott and Professor Qasim Aziz. I forever indebted to them for their motivation, energy, and endless advice that helped me plan my future career in the field of GI physiology.

I should also like to thank other wonderful people that I worked with during my PhD study:

Dr Natalia Zarate *for all her support in the initial studies of colonic manometry and her technical advises; she was one of the most inspirational person by many means;*

Dr Philip Dinning *for his valuable support and knowledge provided for performing and analysing pancolonic manometry studies; I defiantly would not able to progress with colonic manometry studies without the valuable collaboration with his great team in Australia;*

Dr Peter Lunniss *for being my surgical guide, for all his valuable scientific comments and advice to keep my chin up every time I was facing a problem;*

Dr Sean Preston *for scarifying his time to come very early in the morning to do colonoscopies;*

Ms Jacqueline Harbour *for being my support to resolve administrative issues*

Dr Anthony Hobson *for providing clinical data and knowledge for SmartPill analysis*

Polly Rajaram *for her help in cleaning the catheters and tidy up after experiments*

My Parents *for charging my energy every time I feel down and for all their support along my life that make me who I am now;*

Saeed Al-Saadi *for being the most wonderful husband and being patient with me until finished these studies;*

My little boy *for all his destruction and being the best thing happened to me during my study;*

My patients and volunteers *for scarifying their time and being patient to do long studies.*

FUNDING ASSOCIATED WITH THIS THESIS

2009 SmartPill Corporation funded studies described in Chapter 6.

2010 SmartPill Corporation partly funded studies described in Chapter 7.

PUBLICATIONS

Some of the results presented in this thesis have already been published, in part, in the following journals:

PAPERS

1. Caecal pH is a biomarker of excessive colonic fermentation. Farmer AD, Mohammed SD, Dukes GE, Scott SM, Hobson AR. World J Gastroenterol. 2014 May 7;20(17):5000-7.
2. Regional gastrointestinal transit and pH studied in 215 healthy volunteers using the wireless motility capsule: influence of age, gender, study country and testing protocol.Wang YT, Mohammed SD, Farmer AD, Wang D, Zarate N, Hobson AR, Hellström PM, Semler JR, Kuo B, Rao SS, Hasler WL, Camilleri M, Scott SM. Aliment Pharmacol Ther. 2015 Sep;42(6):761-72.
3. Classification of normal and abnormal colonic motility based on cross-correlations of pancolonic manometry data. Wiklendt L, Mohammed SD, Scott SM, Dinning PG. Neurogastroenterol Motil. 2013 Mar;25(3):e215-23.
4. Pancolonic spatiotemporal mapping reveals regional deficiencies in, and disorganization of colonic propagating pressure waves in severe constipation. Dinning PG, Zarate N, Hunt LM, Fuentealba SE, Mohammed SD, Szczesniak MM, Lubowski DZ, Preston SL, Fairclough PD, Lunniss PJ, Scott SM, Cook IJ. Neurogastroenterol Motil. 2010 Dec;22(12):e340-9.
5. Accurate localization of a fall in pH within the ileocecal region: validation using a dual-scintigraphic technique. Zarate N, Mohammed SD, O'Shaughnessy E, Newell M, Yazaki E, Williams NS, Lunniss PJ, Semler JR, Scott SM. Am J Physiol Gastrointest Liver Physiol. 2010 Dec;299(6):G1276-86.
6. Bowel preparation affects the amplitude and spatiotemporal organization of colonic propagating sequences.Dinning PG, Zarate N, Szczesniak MM, Mohammed SD, Preston SL, Fairclough PD, Lunniss PJ, Cook IJ, Scott SM. Neurogastroenterol Motil. 2010 Jun;22(6):633-e176.

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5. 24-Hour Assessment of Pancolonic Motor Activity in Health Using Solid-State Catheter Technology: Comparison With Traditional Water-Perfused Catheters Mohammed SD, Zarate N, Preston SL, Lunniss PJ, Dinning PG, Scott SM. Gastroenterology, Volume 140, Issue 5, Supplement 1, S 1-S-1062
6. Slow transit constipation is associated with an alkaline colonic pH and increased motility: novel findings from studies using the wireless motility capsule. Mohammed SD, Wang YT, Christodoulides S, Semler JR, Hellstrom P, Hobson AR, Dinning PG, Farmer AD, Scott SM. Neurogastroenterol Motil (2015), Vol 27, supp 2, page 87.

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1 BACKGROUND AND LITERATURE REVIEW

1.1. ANATOMY OF THE LARGE BOWEL

The large bowel is also called the ‘colon’. The word (colon) is derived from the Greek word koluein (means “to retard”). The colon is a hollow organ that can vary in length and measures approximately 1.5 metres in length. The diameter of this organ also varies along its length, as it gradually decreases from 7.5 cm at its proximal end to 2.5 cm at its distal end. The large bowel has been subdivided anatomically into: the caecum, ascending colon, transverse colon, descending colon, and sigmoid colon, and the rectum, which lies distally between the rectosigmoid junction and the anal canal. The colon starts proximally at the caecum (Latin word meaning ‘blind’), on the posterior medial wall of which is the appendix within the right iliac fossa. The ileocaecal valve is located at the proximal end of the colon, while the dentate line of the anus is located at its distal end (Figure 1.01).

The ileocaecal sphincter is a circular sphincter originating from the continuous muscular layer of the terminal ileum. Its function appears to regulate the emptying of ileal content, in addition to preventing colonic content refluxing to the ileum.

The dentate line, located at the distal end of the colon, represents the transition between the proximal and distal end of the anus, where the nervous, lymphatic, and blood supplies, and also epithelial lining change.

The ascending and descending colon are attached to the abdominal cavity by their own mesentery, while the transverse colon is partially attached and considered the most mobile part of the colon. The sigmoid colon is the narrowest part of the large bowel and shows variable mobility. The sigmoid colon has its own mesentery, which can be sometimes elongated (dolicolon). The colon also has two flexures along its length: the hepatic and splenic flexures (Figure 1.01).

The colon is wrapped with muscular layers of both longitudinal and circular type, which provide the power essential for propelling bowel contents toward the distal end. The outer longitudinal smooth muscle layer forms three string bundles spaced evenly around the circumference of the colon called the *taeniae coli.* These muscular bundle start at the base of the appendix and extend continuously to the proximal rectum where they fuse into one muscular bundle at the rectosigmoid junction.

One of the exclusive features of the external colonic wall is the presence of

irregularly spaced circumferential constrictions called *haustra,* which spread along its length. Haustra are not fixed structures and appear to move, disappear and then reform continuously during colonic content propulsion; it is believed that both myogenic and neurogenic activities within the colon are responsible for their generation (Cook et al., 2015).

The most distal part of the colon is the rectum. The word rectum is derived from Latin and means 'regular'. Anatomically, the rectum is a curved muscular tube following the inner curvature of the sacrum and measures about 15 cm long; the anus is located at its distal end (Heald and Moran, 1998).

The word anus is also derived from Latin and means 'ring'; it comprises the anal canal and encircling anal sphincters. The internal and external anal sphincters, along with anal vascular cushions act to maintain anal faecal continence during rest (Lestar et al., 1989, Bharucha, 2006). The anal sphincters produce a high-pressure zone (the anal canal), which is usually shorter in women than in men (average 3.7 cm vs. 4.6 cm) and also play an important role in maintaining faecal continence and in the process of defaecation (Irving and Hulme, 1992, Murphy et al., 2007, Bajwa and Emmanuel, 2009). The puborectalis is a U-shaped muscle about 0.5 – 1.0 cm thick, and acts as a flap-like valve that pulls forward the anorectal angle (at approximately 90°, between the long axis of the rectum and long axis of the anal canal) and reinforces it. It also plays a role in maintaining faecal continence at rest and its dysfunction can contribute to faecal incontinence and obstructed defaecation (Bharucha, 2006, Bush et al., 2012, Bajwa and Emmanuel, 2009).

Microscopically, the colonic wall is composed of four layers similar to most parts of the gut: an outer layer called the serosa (adventitia), followed by muscularis (muscularis propria), submucosa, and mucosa (mucosal membrane).

The mucosa is the innermost layer formed by glandular epithelium, lamina propria, and muscularis mucosae.

The mucosa of the colon is lined by a single continuous layer of specialised columnar epithelial cells. Colonic epithelial cells are generated from stem cells located at the base of cylindrical structures called *crypts of Lieberkühn;* these cells migrate toward the intestinal lumen after three to five days on initiation of apoptosis

(Kahn and Daum, 2015) . Other types of cells are also present within the colonic lining, including a large amount of goblet cells (mucus secreting cells) and other secretory cells. The villi (finger-like projections), which are present in the small intestine, are absent in the colon.

The lamina propria supports the epithelium and consists of connective tissues, along with elastin, reticulin, collagen fibres, a network of blood vessels, lymphatics and nerves. The complex connective tissue matrix within the lamina propria contributes significantly to the passive mechanical properties of the gut wall, as it can influence the function of the gut wall mechanoreceptors, in addition to its effects on the function of other neuronal tissues (Grundy et al., 2006).

The muscularis mucosae consists of a thin layer of smooth muscle at the boundary of the mucosa and submucosal layers.

The submucosal layer lies between the thin muscularis mucosae and the muscularis propria. The submucosal layer consists of a fibrous connective tissue layer that contain various structures including fibroblasts, mast cells, blood, lymphatics, and a neuronal plexus (*Meissner's plexus* or *submucosal plexus*).

The muscularis layer is divided into inner circular and outer longitudinal muscle layers. Another neuronal plexus located between these layers is called *myenteric plexus* or *Auerbach's plexus*.

These two neuronal plexuses represent the enteric nervous system and play an integral role in controlling colonic motility (Rae et al., 1998, Huizinga et al., 2009, Sasselli et al., 2012). A group of specialised cells called the *interstitial cells of Cajal* (ICC) also play an important role in controlling gut motor activities and are described as muscle-like cells (Huizinga et al., 2009, Huizinga et al., 2000). In the colon, they can be found in groups surrounding the myenteric plexus throughout the muscle layers, or around non-ganglionated plexuses at the inner border of the gut circular muscle layer (Huizinga et al., 2009). However, the role of these specialised cells in controlling motor activities is better understood in the upper gut than in the colon. These cells act as a pacemaker within the gut wall by generating periodic depolarisations at a constant frequency, which are responsible for generating 'slow wave' motor activities within the gut wall. The consequence of loss, damage, and

death of ICCs can lead to various abnormalities including abnormal gastrointestinal motility and constipation (Porcher et al., 2002, Camilleri, 2001).

The outermost layer of the colonic wall is adventitia or serosa. This layer consists of layers of connective tissue wrapped with a single layer of mesothelial cells.

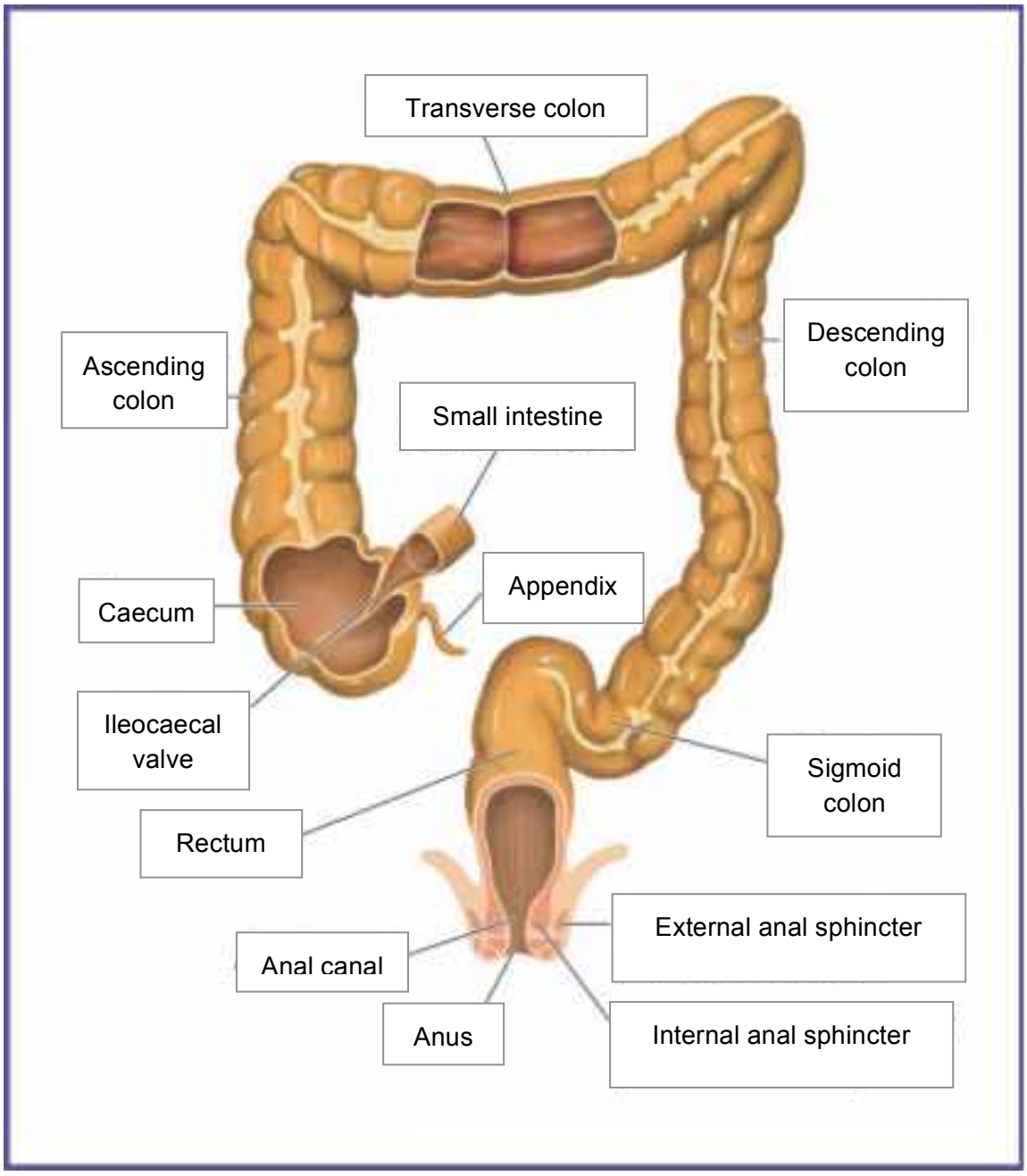


Figure 1.01. Anatomical landmarks of the large bowel.

1.2. NORMAL COLONIC FUNCTIONS

In spite of the fact that the colon is not considered to be one of the vital organs for human life, it is one of the essential parts of the gastrointestinal tract and plays a major role in digestion. Its dysfunction has a high morbidity rate and great socioeconomic impact (Peppas et al., 2008, Everhart and Ruhl, 2009, Nyrop et al., 2007).

The colon subserves 4 main interrelated functions (Scott, 2003):

(1) absorption of water and electrolytes via multiple cellular channels located within the colonic wall. The colon is exposed to 1 - 2 litres of water daily, and can efficiently absorb about 90% of this amount. The colon is able to increase its absorptive capacity to 5 - 6 litres daily (Phillips and Giller, 1973, Debongnie and Phillips, 1978). Diarrhoea results when ileal flow exceeds the maximum capacity of the colon. Electrolytes such as sodium and chloride are also absorbed in the colon.

(2) digestion and fermentation of complex starches and protein by different colonic flora to release energy and various nutrients. The complex compounds are usually resistant to digestion and absorption in the upper gut. In health, the proximal part of the colon contains a higher bacterial density than its distal end which can reach 10^{11} to 10^{12} per gram of colonic content, and comprise more than 1000 bacterial species, the majority of them being anaerobes (Bonfrate et al., 2013, Hamer et al., 2012, Ventura et al., 2009). The bacterial metabolites and other essential micronutrients such as short chain fatty acids, amino acids and some vitamins are absorbed mainly in the proximal colon. These bacterial metabolites are responsible for the energy supply to the colonic cells, as colonic mucosa is unable to extract the nutrients directly from blood (Mortensen and Clausen, 1996, Blottiere et al., 2003).

(3) mixing and propulsion of intraluminal contents, as a consequence of continuous contraction and shortening of colonic longitudinal muscular (taeniae coli) and circular smooth muscle layers; this develops haustral segmentation that aids mixing and propulsive colonic movements.

(4) storage and expulsion of remaining waste products (i.e. defaecation). The sigmoid and the rectum are the main colonic segments that are responsible for intraluminal waste storage.

All of these functions require unique physiological and motor activities of the colon that differ from those of the upper gut. These motor activities include segmentation, propulsion (both in an antegrade and retrograde direction), changes in muscular tone and complex co-ordinated contraction/relaxation movements between the colon, rectum and anus. Furthermore, colonic contents may remain *in situ* for many hours or even days to ensure proper mixing and absorption, and thus prolonged investigation (24 hours or more) is needed to assess the various aspects of colonic motor function (motility) (Scott, 2003).

1.3. THE PROCESS OF DEFAECATION

Defaecation is a complex process and not fully understood. Normal defaecation is a co-ordinated process involving the central, autonomic and enteric nervous systems, and requires integrated activities of normal colorectal motility, anorectal sensation and sufficient expulsive forces through a relaxed but supportive pelvic floor. The average stool output of an adult of western origin is about 200 grams daily (Irving and Catchpole, 1992), of which 65 – 85% is water. This is greatly determined by the absorptive capacity within the proximal colon that affects the consistency and volume of bowel content. Conversely, the rectosigmoid acts as a reservoir for bowel contents. However, as rectal filling gradually increases along with increase in intrarectal pressure, a subconscious perception of the nature of rectal content is initiated. An intact anal sphincter complex helps to ensure bowel continence until voluntary defaecation is possible and socially acceptable. In addition, normally, intra-anal pressure is higher than the intrarectal pressure, which also helps maintaining faecal continence at rest. Generally, the defaecation process can be divided physiologically into four phases: (1) the basal phase, (2) a pre-defaecatory phase (defaecatory urge), (3) the expulsive phase or evacuation, (4) termination of defaecation (Palit et al., 2012).

At rest, the pelvic floor muscles including the levator ani, puborectalis, and the external anal sphincter are all contracted by the effect of the 'postural reflex' (Porter, 1962), which provide extra support to the pelvic organs. Defaecation begins with rectal sensory perception of a critical level of rectal filling, which is relayed as a need to evacuate the rectum (Palit et al., 2012). The actual volume that triggers this perception is dependent on the biomechanical properties of the rectum and its contents (a broad description of rectal sensory function is beyond the scope of this thesis). Briefly, rectal sensation originates from stimulation of nerve endings and mechanoreceptors within the rectal wall and adjacent structures, and is transmitted mainly by pelvic splanchnic, S2 - S4 parasympathetic nerves and spinal afferent neurons located within the lumbar and sacral dorsal root ganglia (Brookes et al., 2009). At the same time, rectal filling activates afferent autonomic neurons that result in both conscious perception and activation of local reflexes (e.g. the anal sampling reflex) to begin the relaxation of the internal anal sphincter along with voluntary contraction of the external anal sphincter (Miller et al., 1988). The anal sampling reflex allows the anorectal content to be 'sampled' after each filling period and discrimination as to the nature of rectal content occurs (i.e. solid, liquid, gas). This reflex can be induced in the clinical setting by artificial rectal distension (named the recto-anal inhibitory reflex, RAIR) (Palit et al., 2012), which was first described by Gowers in 1877 (Gowers, 1877) and by Denny-Brown *et al* in 1933 (D. Denny-Brown and Robertson, 1933;). When the situation is appropriate, the individual adopts a sitting or squatting position that results in opening of the anorectal angle that allows more effective expulsion of the rectal contents. During this process, abdominal wall muscles tense, resulting in an increase of intra-abdominal and intra-pelvic pressure; concomitant relaxation of the pelvic floor then pushes the stool to the lower rectum (Palit et al., 2012). As a consequence, spontaneous recto-sigmoid contractions will initiate further propulsion of the stool through a relaxed anal canal. Large propulsive contractions within the colon and rectum begin to increase 1 hour before stool expulsion (Bampton et al., 2000). Research and clinical studies of colonic contractile activities, along with radiographic and scintigraphic studies, show that propulsive activities originate in the proximal colon before and during defaecation; these have the ability to empty a great proportion of colonic contents (Lubowski et al., 1995, Halls, 1965). Bampton *et al*. showed in healthy volunteers that a spatial and temporal relationship among propagating contractile sequences originating from different sites

occurs within the colon in the pre-defaecatory phase. They also demonstrated a stereotypic anal followed by orad migration, which raises the possibility that defaecation is controlled through long colo-colonic pathways (Bampton et al., 2000).

1.4. CONSTIPATION

1.4.1. INTRODUCTION

Constipation is a Latin word (constipatio) meaning crowding together. Constipation is a very common symptom-based disorder. Traditionally it has been considered as one of the non-organic or 'functional' gastrointestinal disorders that embrace a number of symptoms, which describe an individual's personal difficulties with and/ or infrequency in emptying bowel content. There are various definitions and terms that describe constipation; some rely on symptoms volunteered by the patient (*symptom*-based classification) (Rome, 2006), including scoring systems based on symptoms frequencies (Agachan et al., 1996, Knowles et al., 2000, Slappendel et al., 2006, Renzi et al., 2013), and others which depend on *measurement*-based classification (described below in detail in section 1.4.6). A detailed review of symptom-based classification and scoring systems is beyond the scope of this thesis.

In general, most constipation sufferers respond well to over-the-counter medications and conventional treatments, including dietary modification (e.g. increased fibre intake), change in lifestyle, and use of laxatives. The majority of such sufferers rarely present to secondary or tertiary medical services, and either self-treat or are managed by their general practitioners. However, the pathophysiology of what is considered a 'colonic motility disorder' remains elusive. This is certainly important in those patients with intractable symptoms, where detailed colorectal physiological tests are indicated to determine the cause of colonic and anorectal dysfunction, in order to better target available and novel therapeutic strategies (both medical and surgical). However, the relative merits of symptom-based versus measurement-based definitions to sub-classify patients with constipation into more homogenous groups, remains hotly debated (Cook et al., 2009).

The rationale behind this is that constipation is a disorder that embraces heterogeneous aetiologies and in order to define underlying pathophysiologies and

to better target therapies, sub-classification of such patients is very important. Unfortunately, there remains a lack of specific biological and/or physiological markers for chronic intractable constipation that are able to identify underlying pathophysiology and help to direct therapeutic strategies and to predict their therapeutic outcomes.

1.4.2. EPIDEMIOLOGY AND SOCIOECONOMIC BURDEN

Constipation is one of the most common gastrointestinal symptoms volunteered by adults and children (van den Berg et al., 2005). In fact, constipation is the second most commonly self-reported gastrointestinal symptom after dyspepsia, affecting around 15% of adult populations (Stewart et al., 1999, McCrea et al., 2009a, McCrea et al., 2009b, Ferrazzi et al., 2002, Peppas et al., 2008). Constipation appears to be more common in boys in childhood, while women are the main sufferers during adulthood (Higgins and Johanson, 2004, van Ginkel et al., 2003, van den Berg et al., 2005, Iacono et al., 2005, McCrea et al., 2009a). Furthermore, a recent review of 11 studies examined the prevalence of constipation in various age groups, and showed that constipation rates appear to increase gradually after the age of 50 (3% - 28.4%), and is more evident after the age of 70 (8% - 43%) (McCrea et al., 2009b).

Epidemiological studies show wide variation in reported prevalences of constipation of between 2 - 28% (Sonnenberg and Koch, 1989, Everhart et al., 1989, Talley et al., 1991, Talley et al., 1993, Stewart et al., 1999, Pare et al., 2001, McCrea et al., 2009b); this variation is dependent on multiple factors such as gender, age, socioeconomic status, educational level, and race (Ludvigsson, 2006), and most notably the instrument (i.e. questionnaire) used to derive whether constipation is present or absent (Wald et al., 2008). Functional gastrointestinal disorders including constipation are associated with significant economic (Rantis et al., 1997) and lifestyle (Sailer et al., 1998) impact due to their chronicity. Total health care costs for patients suffering from constipation in the USA alone during 2004 amounted to $1.7 billion (Everhart and Ruhl, 2009). In fact, total healthcare costs are reported to be higher for patients suffering from functional constipation compared to those with the irritable bowel syndrome (Nyrop et al., 2007).

1.4.3 CLINICAL PRESENTATION

Constipation is a very heterogeneous disorder; patients can present with a variety of symptoms including those directly related to the defaecation process, for example; infrequency of bowel opening, passage of hard stool, loss of urge to defaecate, straining, sense of incomplete evacuation, painful defaecation, blockage sensation or unsuccessful evacuatory attempts (Gastroenterology., 2005, Rome, 2006). Symptom frequency and severity may fluctuate over time. Furthermore, some constipated patients can intermittently present with bouts of loose stool, probably secondary to laxative use (Dinning et al., 2011) or faecal impaction and overflow diarrhoea (Read et al., 1985, Loening-Baucke and Cruikshank, 1986, Scarlett, 2004, Wald, 2005). Patients may also volunteer more diverse gastrointestinal and extra gastrointestinal symptoms such as abdominal pain, bloating (which can be associated with visible abdominal swelling), back pain, and/or nausea. Very commonly, particularly in paediatric and geriatric populations, there is co-existent faecal incontinence, which is generally considered secondary to underlying constipation. In adult populations, there is evidence to show constipation and incontinence also co-exist (Mohammed et al., 2010, Nurko and Scott, 2011). Without clear definition of what defines normality or abnormality in terms of the frequency of symptoms reported by patients, the definition of constipation remains highly subjective.

1.4.4 AETIOLOGY OF CHRONIC CONSTIPATION

Simple constipation is very common, and the majority of cases are related to a sedentary lifestyle and poor fluid and fibre intake. This can easily be reversed with lifestyle and dietary modification. Nevertheless, a wide range of organic disorders can affect colonic function, and as a consequence patients may present with symptoms of constipation (Locke et al., 2000, Jamshed et al., 2011) (Table 1.01). In general, patients can be divided into those for whom routine clinical investigations, including biomedical laboratory tests, and radiological and endoscopic examinations, define a cause for their constipation (termed *secondary constipation*) and those in whom such tests are normal. This group tend to be labelled as **idiopathic / primary / or functional constipation**.

1. GASTROINTESTINAL CAUSES

a. Colorectal	Mechanical obstruction	Benign and malignant
		other strictures: inflammatory
	Megacolon / megarectum	Hirschsprung's disease
		Idiopathic
		Neurological or other
b. Anorectal	Congenital malformation	
	Hereditary internal anal sphincter hypertrophy	
	Anal stenosis	
	Rectal prolapse	
	Large rectocoele, obstructing intussusception	
	Haemorrhoids and fissures	
	Solitary rectal ulcer syndrome	

2. EXTRAGASTROINTESTINAL CAUSES

a. Endocrine	Hypothyroidism
	Hyperparathyrodisim
	Diabetes mellitus
b. Metabolic	Hypercalcaemia, Hyperkalemia, Hypermagnesia
	Porphyria
	Uremia
c. Neurological	Cerebrovascular diseases (e.g. Multiple sclerosis, Parkinson's)
	Spinal cord injury
	Damage to sacral parasympathetic nerves
	Autonomic neuropathy
	Paraplegia
d. Psychological	Severe depression
	Eating disorders (e.g. anorexia)
e. Connective tissue disorders	
(e.g. Scleroderma, Amyloidosis, Ehlers-Danlos Syndrome [Hypermobility type])	
f. Drugs	opiates and narcotics, anticholinergics, antidepressants, anticonvulsants, antacids, diuretics, calcium channel blocker, iron supplement, non-steroidal anti-inflammatory drugs

Table 1.01. Summary of main gastrointestinal and extra-gastrointestinal organic causes of chronic constipation (Rao and Go, 2010, Locke et al., 2000, Schiller, 2001, Prather and Ortiz-Camacho, 1998)**.**

1.4.5. CLINICAL INVESTIGATION OF CHRONIC CONSTIPATION

After routine clinical examination (including abdominal and digital rectal examination), which may reveal the presence of faecal loading, abdominal x-ray and sigmodioscopic examination are performed; however, the findings are frequently unremarkable. Routine blood tests should also be performed to exclude any underlying endocrine or metabolic cause of constipation, although tests are rarely positive.

A variety of complementary investigations exist for the assessment of anorectal structure and function with reference to continence and evacuation efficacy. However, the number of physiological investigations performed and their protocols may vary considerably between performing centres.

1.4.5.1. Rectal sensory testing

The importance of rectal sensory dysfunction is increasingly recognised in functional bowel disorders, including chronic constipation (Gladman et al., 2003). Different stimuli can be applied to the rectum, such as mechanical, thermal, and electrical. However, mechanical distension is the most reliable and clinically well-accepted method of assessment of sensation (Scott and Gladman, 2008), and is considered to be the most physiological, as it is intended to mimic rectal filling.

Rectal sensation can be simply evaluated using a *latex balloon*, secured to a catheter, and inflated with air or water at a rate of 1 ml/sec, in order to determine threshold volumes for first constant sensation, defaecatory desire and maximum toleration (Farthing and Lennard-jones, 1978). This test is the most convenient method in everyday clinical practice. Values obtained for each sensory threshold can be compared to normal ranges matched for age and gender (Jameson et al., 1994). However, the main limitation of this test is the confounding nature of the elastic properties and compliance of the balloon itself. Furthermore, the test is unable to differentiate between altered rectal sensation secondary to neuronal damage or due to change in rectum calibre (megarectum) and compliance.

The **electromechanical barostat** is a more advanced technique by which to assess rectal sensorimotor and biomechanical function. In general, an initial conditioning distension is recommended to precede any distension protocol, to obtain a stable basal tone, and to familiarise the subject with the sensation resulting from barostat bag inflation (Hammer et al., 1998). The minimum distention pressure (MDP) is also determined; this is defined as the minimum pressure at which changes in rectal bag volume associated with respiratory movements are observed. This therefore represents the minimum pressure required to distend the barostat bag but without inducing rectal wall deformation (Serra et al., 1998, Gladman et al., 2005). The barostat is the gold standard for measuring rectal sensory perception (Burgell and Scott, 2012). Sensory thresholds and stimulus intensity assessments are two parameters used to measure rectal sensation by this technique (Bharucha et al., 2004). A sensory threshold is measured by applying gradual distension to the rectum with stepwise increases in pressure over time. The subject is asked to report their first constant sensation, urge threshold and maximal toleration (Whitehead and Delvaux, 1997).

Two distension protocols are commonly used to assess rectal sensation: (1) an ascending method of limits, where volume or intra-rectal balloon pressure are progressively increased in a phasic, stepwise, or ramp manner (Figure 1.02); (2) random phasic distention, where the subject is exposed to randomly selected phasic distensions selected by the computer or the investigator, which has the advantage of limiting sensation biases. Stimulus intensity assessment is performed by asking the subject to rate intensity on a visual analog scale (Flaherty, 1996, Hjermstad et al., 2011). There are many factors that can influence barostat results, such as fasting status, subject position, rectal content, anatomical variation, barostat bag size and compliance, and also barostat inflation protocol (Whitehead and Delvaux, 1997). In addition, the presence of proctitis, spinal cord injury, and previous rectal surgery can also interfere with the results of a barostat study (Putta and Andreyev, 2005, Matzel et al., 1997, Rasmussen et al., 2003, Bharucha et al., 2005). Given different distension protocols, there is difficulty in standardising the technique and comparing results between centres, though guidelines have been published (Whitehead and Delvaux, 1997).

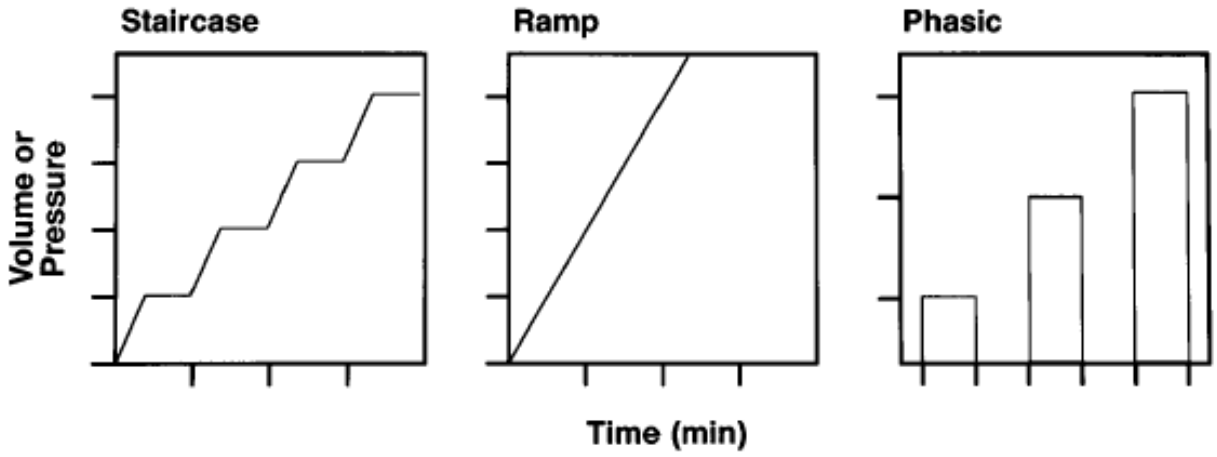


Figure 1.02. Representation of ascending method of limits of barostat distension. For staircase and ramp protocols, the amount of air within the barostat bag continually increases up to the maximum level. For phasic distensions following each specified volumetric distension, deflation of the balloon is performed within a constant time frame.

1.4.5.2. Assessment of rectal evacuation

Different methods are available to assess rectal evacuation and pelvic floor structure during the process of defaecation. However, none are standardised and normative data are lacking. Tests that are commonly performed include:

1.4.5.2.1. Anorectal manometry

Manometry is the method of recording mechanical activity of the gastrointestinal tract through the measurement of changes in intraluminal pressure. Anorectal manometry is a good, reliable test within the same laboratory, and can help to predict the outcome of clinical management (Cook et al., 2009).

In chronic constipation, anorectal manometry is used as a tool to assess dyssynergic defaecation (lack of co-ordination of recto-anal function, resulting in anal pressure remaining higher than rectal pressure) (Rao, 2008), often in combination with the balloon expulsion test. However, under laboratory conditions, patients may not produce normal anal relaxation, which may lead to an over-diagnosis of this condition (Lunniss et al., 2009).

The diagnostic yield of anal manometry for dyssynergic defaecation is reported to be 30 - 75% (Rao et al., 2005) and based on manometric findings, Rao has attempted to sub-classify dyssynergic defaecation into four subcategories (Rao, 2008) (Figure 1.03):

Type 1: paradoxical increase in anal sphincter pressure, in the presence of an adequate pushing force and rise in intra-abdominal pressure;

Type 2: paradoxical anal contraction, in the absence of an adequate pushing force (but no increase in intrarectal pressure);

Type 3: presence of adequate pushing force but either has absent or incomplete sphincter relaxation;

Type 4: absence of an adequate pushing force and absent or incomplete anal sphincter relaxation.

Nevertheless, the significance of such proposed classification in term of management is unclear.

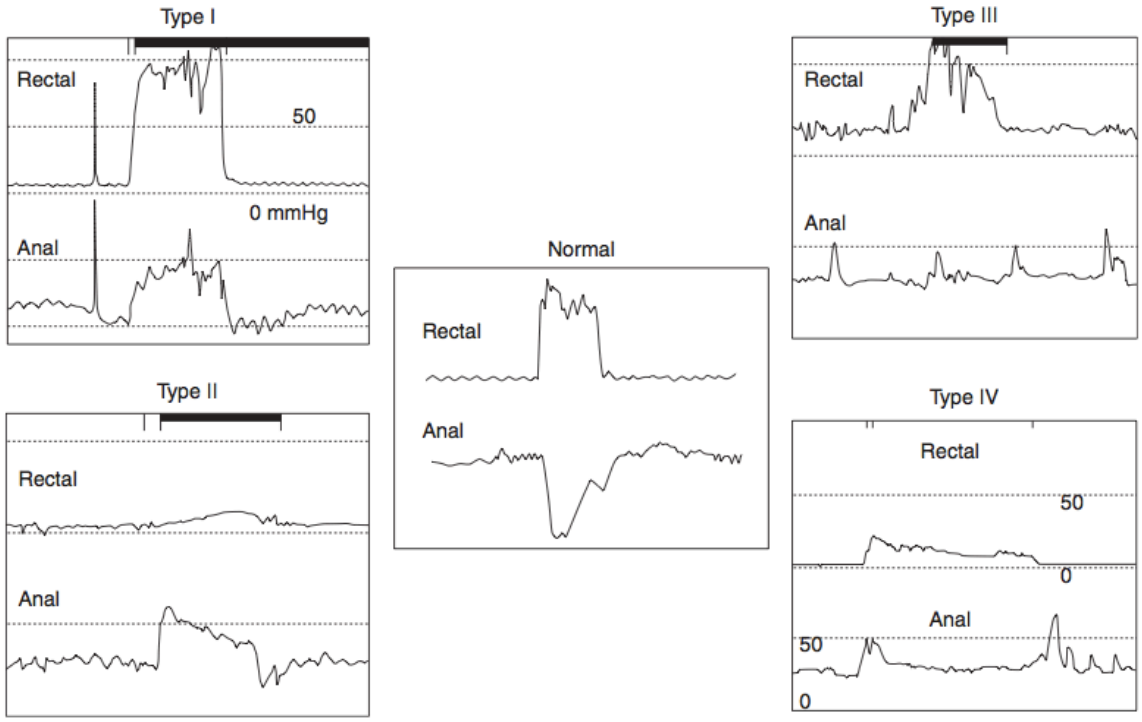


Figure 1.03. Schematic representation for manometric patterns of dyssynergic defaecation (Rao, 2008).

1.4.5.2.2. Balloon expulsion test

This is a simple test to evaluate a patient's ability to expel a filled balloon from the rectum. The balloon is usually filled with 50 ml of warm water or air and the subject is asked to evacuate the balloon in either a left lateral position, seated on a commode, or with the aid of external traction (Barnes and Lennard-Jones, 1985) or more recently advised to be in a sitting position (Bharucha and Wald, 2010, Rao et al., 2006). The balloon may be connected to a manometric catheter to record recto-anal pressure changes. In normal subjects, balloon expulsion usually occurs within 1 minute (range 10 – 300 second) (Rao et al., 1999). The test is a useful screening test for a functional defaecation disorder, but it does not define the mechanism of disordered defaecation (Scott and Gladman, 2008). Most importantly, the test does not provide any information about any structural abnormalities that may affect the process of rectal evacuation.

1.4.5.2.3. Evacuation proctography (or defaecography)

This is a radiological test to assess morphological information and to record the dynamic movement of the pelvic floor and anorectal anatomy during rest, coughing, squeezing, and straining after instilling barium contrast (neostool) into the rectum (Womack et al., 1985). Wallden first described this method in 1954 for diagnosing outlet obstruction (Wallden, 1954) but the test become more popular in the 1980s (Mahieu et al., 1984b, Mahieu et al., 1984a). The test has the advantage over balloon expulsion in providing anatomical information (Lunniss et al., 2009). The amount of evacuated stool and the time taken to evacuate are recorded. In addition, other parameters are usually assessed, such as rectal diameter at rest (to evaluate the possibility of megarectum), recto-anal angle change, anal sphincter relaxation, and obstructive morphological features (e.g. rectocoele or intussusception).

Proctography is usually performed without prior bowel preparation. The patient lies in the left lateral position and a previously prepared semisolid barium contrast paste (neostool) is instilled into the rectum via a large bore syringe with the aid of proctoscope (Goei et al., 1989, Chan et al., 2001, Zarate et al., 2008). The amount of instilled neostool can be a fixed amount (usually 120 ml) or a volume individualised to each patient based on the sensation of

sustained desire to defecate (Goei et al., 1989, Freimanis et al., 1991). The use of an individualised volume improves the ability to evacuate during the test (Chan et al., 2001, Gladman et al., 2006, Palit et al., 2012). The subject is then transferred to a radiolucent commode on the x-ray table, and lateral fluoroscopic images are taken at rest and during attempted defaecation.

Limitations of this technique include: the difficulty in interpreting the clinical significance of anatomical anomalies, given the large overlap between health and defaecatory disorders. For example, small rectocoeles and intussusceptae are found frequently in subjects without defaecatory symptoms (Shorvon et al., 1989). Nevertheless, large rectocoeles and obstructing intussusceptae do impede evacuation and are consistent with symptoms of evacuatory difficulty (Dvorkin et al., 2005). The test is also limited by subject embarrassment, which may prevent patients emptying their bowel content as under normal circumstances. Furthermore, the semisolid contrast used may not mimic normal rectal contents, such as in the case of patients with constipation allied to hard stools.

1.4.5.2.4. Dynamic magnetic resonance imaging (MRI) defaecography

This test was introduced in 1991 and can also be useful for the assessment of anorectal function and evacuation efficacy (Kruyt et al., 1991). It has the ability to evaluate pelvic floor anatomy (including the anterior, middle, and posterior compartments) and its dynamic motion simultaneously (Rentsch et al., 2001, Seynaeve et al., 2006). It has the advantage over conventional defaecography in that radiation exposure is avoided and therefore it can be repeated if needed. It also has better resolution and excellent soft tissue imaging (Seynaeve et al., 2006). It can be used to clarify the underlying pathophysiology of many complex pelvic floor disorders such as enterocoele, sigmiodocoele, and utero-vaginal prolapse. The main limitation of this technique is that patients are generally required to be in the supine position (not physiological), which can influence the structural anatomy and pelvic physiology during defaecation. The procedure is also poorly standardised and there is a lack of normative data.

1.4.5.3. Assessment of colonic transit

Colonic transit time (CTT) is defined as the time required to clear intracolonic food contents from the point of entry in the caecum, until its exit from the anal canal (by the process of defaecation). Measurement of CTT is essential for patients presenting with chronic idiopathic constipation. Various methods have been used in an attempt to measure colonic transit (for more details, see section 1.6.1.2). Two techniques currently used in routine clinical assessment of colonic (or whole gut) transit; both are involve radiation exposure: radio-opaque markers (ROM) and colonic scintigraphy (for more details, see section 1.6.1.2). Transit studies using ROM is widely available in clinical practice and is first described by Hinton *et al* in 1969 (Hinton et al., 1969). ROM test is considers as a screening test of delayed colonic transit. ROM are small solid particles; they are usually made from barium-impregnated polyvinyl chloride material. Patients ingest one or more capsules contained these markers and a plain abdomenopelvic X-ray use to assess their movement through the GI tract (Figure 1.06). Previous studies showed that the upper limit of normal of CTT is around 70 h (Dinning et al., 2009a). Clinically, retention of >20% of markers as determined by the X-ray is indicative of slow transit constipation (see below, section 1.5). However, the components of this test are not standardised specifically: number of ingested markers, shapes of markers, duration of study, and number and timing of X-ray images required (for more details, see section1.6.1.2.1).

1.4.6. MEASUREMENT-BASED CLASSIFICATION OF CHRONIC CONSTIPATION

After routine investigation to exclude an organic or dietary cause of symptoms, a sub-group of patients with chronic intractable problems will be offered specialist referral for further physiological investigation. At present, colonic transit and rectal evacuation are the main components that are usually measured (Longstreth et al., 2006, Locke et al., 2000, Rao, 2007), the results of which are used to sub-classify patients with chronic constipation into 3 main categories:

1. **Normal transit constipation:** this term is mainly used in the USA, and is based on normal findings of a colonic transit study in addition to normal rectal balloon expulsion. However, the presence of rectal structural abnormalities using defaecography has not been considered, nor rectal sensory dysfunction.

2. **Slow transit constipation (STC):** those patients in whom there is mainly a reduction in the propulsive capacity of all or part of the colon currently based on an assessment of colonic transit using ROM and/ or scintigraphic assessment.

3. **Evacuatory disorders (ED**): those in which there is predominantly a disorder of rectal evacuation based on impaired rectal balloon expulsion and/ or abnormal manometry (notably in North America) or defaecography (particularly in Europe).

These subtypes commonly coexist or overlap within the same patient (Cook et al., 2009); however, there is considerable discrepancy as to their reported prevalences, which likely reflects methodological variation and also population variation. Nyam *et al* examined the prevalence of STC and ED in a large cohort of patients (n=1009) referred to one centre; they reported that 59% had normal tests results, 25% had ED, 13% had STC, and only 3% had both STC & ED (Nyam et al., 1997). In the same year, using a different method to assess evacuation (defaecography), Koch *et al* investigated the presence of transit delay and evacuatory dysfunction in 190 constipated patients. They reported higher prevalences of STC and ED compared to the previous study (only 8% had normal test results, 59% had ED, 27% had STC only, and 6% had both) (Koch et al., 1997). In both studies, ROMs were used to investigate for transit delay although some patients in the Nyam *et al* study also

underwent scintigraphic assessment. Nevertheless, STC and ED are also known to be heterogeneous disorders, as other pathophysiological findings can coexist themselves, such as a disturbance in rectal sensation (i.e. rectal hyposensitivity, found in a quarter of constipated patients (Gladman et al., 2003). Nevertheless, a small group of patients will have normal colonic transit and normal parameters of rectal evacuation in the presence of constipation symptoms, which raises the question of the presence of as yet unidentified underlying pathophysiologies, which may require alternative physiological investigation and/or clinical assessment. Furthermore, the measurement of colonic transit (which defines STC) is itself only an indirect indicator of the organ's motor status. *Altered colorectal motor activity* represents one of the principal and well-accepted hypotheses to explain chronic functional constipation**;** this requires direct study of colonic motor function. However, only a few centres worldwide are involved in the direct study of hindgut motility, and consequently, there is a relative paucity of data regarding *normal* colonic motor function, which is absolutely fundamental to defining abnormalities in gut dysmotility disorders. Although much progress has been made, we currently remain unable to classify adult patients according to their colorectal motor function, which can delineate patients into more homogenous groups.

1.5. SLOW TRANSIT CONSTIPATION (STC)

1.5.1. DEFINITION

STC is a physiological diagnosis reflecting prolonged progress of colonic intraluminal contents as defined by colonic transit studies. The term, STC, was first described in the 1980s in a group of women who all displayed slow total gut transit time with a normal calibre bowel (Preston and Lennard-Jones, 1986). STC can exist throughout all colonic regions (generalised transit delay), be limited to a specific colonic region (regional transit delay) or indeed be part of a pan-enteric disorder affecting the whole GI tract. The majority of STC cases are considered idiopathic; however, some clear systemic causes are evident, such as defined neuronal damage (Valles and Mearin, 2009). More than 100 years ago, the most severe form of STC was described as 'chronic intestinal stasis' (Lane, 1908, Lane, 1909). Acute (Ogilvie's syndrome) and chronic colonic pseudo-obstruction also represents severe forms of STC, which are usually associated with abnormal colonic calibre (*mega*colon: increase in maximum colonic diameter) and require urgent medical and surgical intervention (Kamm, 2000, Emmanuel et al., 2004, Durai, 2009).

1.5.2. EPIDEMIOLOGY

It is difficult to measure the true prevalence of STC, as the majority of patients with constipation do not have transit studies. STC is reported to be present in 3 - 37% of patients presenting with severe constipation (Sonnenberg and Koch, 1989, Johanson and Sonnenberg, 1994, Surrenti et al., 1995, Nyam et al., 1997, Koch et al., 1997, Stewart et al., 1999). Rao *et al* reviewed 10 studies of colonic transit testing and showed a prevalence of 38 to 80% for STC (Rao et al., 2005).

A higher prevalence rate of STC was recently reported within one of the tertiary motility centres in America with 42% found to have isolated STC and 25% combined with evacuation dysfunction (secondary to dyssynergic defaecation) out of 212 patients presenting with STC (Shahid et al., 2012). The wide range in reported prevalences is mainly due to different definitions and diagnostic criteria and investigation methods used to determine colonic transit times.

In addition, higher prevalences tend to be reported from tertiary centres where most

of the severe cases of constipation are referred and multiple investigations often performed.

1.5.3. STC AND GENDER VARIATION

Longer colonic transit times have been reported in healthy females compared to men. Chronic constipation is also reported to be more common in females (McCrea et al., 2009b). Similarly, STC mainly affects females during their productive age (Preston and Lennard-Jones, 1986, Roe et al., 1988, MacDonald et al., 1993, Knowles and Martin, 2000). This gender variation has been assumed to be related to the level of female sex hormones (mainly progesterone), as constipation symptoms are also often reported to be most severe in the 2nd and 3rd trimesters of pregnancy, when the levels of progesterone are at their peak (Everson, 1992, Baron et al., 1993). Furthermore, colonic transit times may be longer in the luteal phase of the menstrual cycle compared to the follicular phase (Jung et al., 2003). Nevertheless, the blood levels of progesterone have been shown in other studies to be normal in severely constipated patients (Kamm et al., 1991). However, a recently series of *in vitro* studies performed by Behar *et al* on human colonic tissues of STC patients showed that progesterone receptors and their regulatory enzymes are overexpressed in colonic muscles, possibly resulting in tissue sensitization to normal levels of the hormone; this may affect colonic muscle contraction and relaxation (Xiao et al., 2005, Cong et al., 2007, Cheng et al., 2008, Cheng et al., 2010, Guarino et al., 2011).

1.5.4. NATURAL HISTORY OF STC

In patients with STC, the majority of cases arise *de novo* in early childhood, and are labelled as chronic and idiopathic (Preston and Lennard-Jones, 1986). However, many patients may only volunteer their symptoms at later stages in life when symptoms become more severe and interfere with their daily activities. The aetiology of such idiopathic cases remains unclear, and is probably itself heterogeneous (Knowles et al., 1999b). Symptom onset in some of these patients will follow events such as hysterectomy (Roe et al., 1988, Smith et al., 1990, MacDonald et al., 1993) or childbirth (MacDonald et al., 1997). In a previous study, the differences in colonic transit between these separate clinical subgroups had been highlighted, and demonstrated a positive correlation between severity of transit abnormality as

determined by colonic scintigraphy and duration of symptoms in patients with chronic idiopathic STC but not STC secondary to pelvic trauma (Figure 1.04) (Scott et al., 2001). This study by Scott et al concluded that STC might be a progressive disorder, which is consistent with clinical findings. Unfortunately, there is a paucity of data available in terms of the natural history of STC and other types of chronic constipation. Such information is fundamental to understand expected disease progression, which may be important in determining the timing and method of medical and/or surgical intervention.

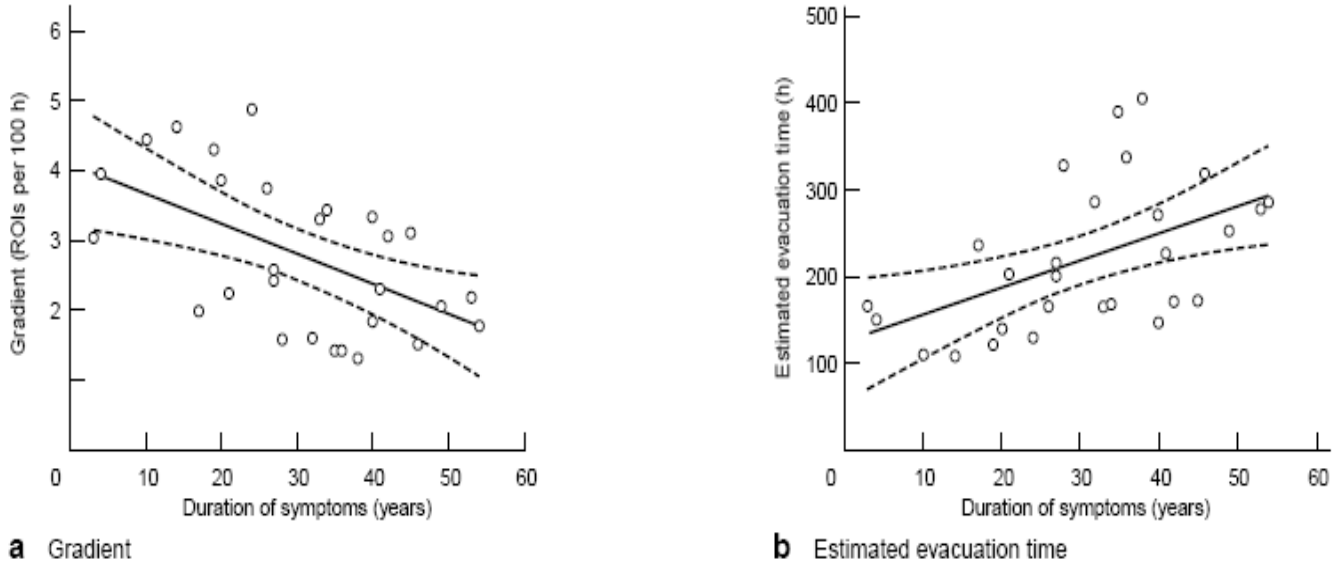


Figure 1.04. The effect of duration of symptoms on (a) gradient of geometric centre of isotope mass (GCI) progression and (b) estimated evacuation time in patients with chronic idiopathic slow-transit constipation (STC) and generalised pattern of transit delay. Both variables are reliant on spatial progression of GCI over the entire study period for each subject with a generalised chronic transit delay. Regression lines (solid) are shown with 95 per cent confidence intervals (broken lines). ROI: region of interest. **a** $r^2 = 0.30$, $P = 0.003$; **b** $r^2 = 0.26$, $P = 0.005$ (Scott et al., 2001).

1.5.5. EVACUATORY DYSFUNCTION AND STC

1.5.5.1 Definition of evacuatory dysfunction

The term evacuatory dysfunction (ED) embraces symptoms that describe a patient's dissatisfaction with their defaecation (Lunniss et al., 2009). Various terms had been described under the umbrella of ED such as: outlet obstruction, obstructed defaecation, dyssynergic defaecation, anismus, pelvic floor dyssynergia and puborectalis dyssynergia; however, these terms may relate to specific underlying mechanisms (Lunniss et al., 2009). Symptoms of ED include: incomplete rectal emptying, sensation of anorectal obstruction and manual rectal evacuation (Rome, 2006). In fact, most patients with chronic constipation primarily complain of symptoms of ED.

1.5.5.2. Epidemiology

Little is known with regard the true prevalence of ED in patients suffering from chronic constipation including STC. It had been reported to be more common in women than men (Everhart et al., 1989, Pare et al., 2001). Variable prevalences had been reported range from 3.5% - 19% (Stewart et al., 1999, Jelovsek et al., 2005, Varma et al., 2008). This variation is due different criteria used to describe ED.

1.5.5.3. Pathophysiology of evacuatory dysfunction

Various underlying pathophysiological abnormalities have been described in patients with ED with or without slow colonic transit.

1.5.5.3.1. Disturbances of rectal sensory function

An elevated sensory threshold to rectal volumetric distension is known as 'rectal *hypo*sensitivity' (RH), which has been reported in various lower gut disorders including constipation and faecal incontinence. The prevalence of RH in constipation is between 18% and 68% (Gladman et al., 2006). However, there is still no clear understanding of the pathoaetiology of RH. It is strongly believed that damage to the rectal afferent neuronal pathway within the brain-gut axis can induce blunted rectal sensation (Burgell and Scott, 2012). This neuronal damage can occur secondary to well documented causes such as pelvic nerve injury and spinal trauma (Nakai et al., 2000, Gladman et al., 2003). Generally, RH can be subdivided into 'primary' RH and 'secondary' RH, which is associated with altered rectal biomechanical properties (i.e.

secondary to increase in rectal capacity or increased rectal compliance' (Burgell and Scott, 2012). RH has been well documented in a proportion of patients with STC (Read et al., 1986, Waldron et al., 1988, Kamm and Lennard-Jones, 1990, Gladman et al., 2003). Whether the presence of RH influences transit and motor activities differently to those suffering from isolated STC is unknown.

1.5.5.3.2. Abnormal biomechanical properties

In patients with documented STC, published studies show inconsistent findings in terms of rectal tone and compliance. Several studies evaluating women with symptoms of ED and STC using the electromechanical barostat have reported attenuated or blunted rectal tone following mechanical distention, administration of a bisacodyl, and after physiological stimuli (ingestion of a meal) (Schouten et al., 1998, Gosselink et al., 2000, Gosselink et al., 2001). Similar findings have been reported in a small number of females with STC but without symptoms of ED (Grotz et al., 1993). Conversely, Felt-Bersma *et al* reported that patients with constipation appear to have normal compliance and rectal tone (Felt-Bersma et al., 2000). Other studies have reported an increase in rectal compliance, which has been attributed to excessive laxity and loss of resistance to distension, suggesting abnormal biomechanical properties (Gosselink et al., 2001, Gladman et al., 2005). Intestinal wall connective tissue, containing collagen, along with smooth muscle activity are responsible for the passive properties of the gut wall which contributes to compliance (Gregersen and Kassab, 1996).

Little is known about colonic tone and compliance measured by the barostat in chronic constipation or STC. One study showed that colonic fasting and postprandial tone were normal in STC compared to those suffers from ED (Ravi et al., 2010).

1.5.5.3.3. Structural/ functional abnormalities

ED symptoms may be attributed to other structural anatomical abnormalities within the rectum (e.g. rectocoele) (Turnbull et al., 1988, Wald et al., 1990, Karlbom et al., 1995), or 'functional' disturbance, such as paradoxical puborectalis contraction or pelvic floor dyssynergia (Turnbull et al., 1986, Shouler and Keighley, 1986, Wald et al., 1990, Miller et al., 1991). Such abnormalities have been well documented in STC (Turnbull et al., 1986, Wald et al., 1990, Miller et al., 1991, Wald et al., 1993, Karlbom et al.,

1995). The influence of specific abnormalities on pattern of transit delay or other colonic motor abnormalities in STC is unknown.

1.5.6. MANAGEMENT OF STC

1.5.6.1. Available therapies

A. Pharmacological

After attention has been given to changes in lifestyle, dietary and fluid intake, as well as change in stooling or toileting behaviour (similar to other types of constipation), laxatives are usually consider to be effective as short-term medications for treating STC. These include:

1. *Bulking agents,* these are polysaccharides, which act by increasing water content within bowel contents. However, they are not very effective in patients suffering from STC as, for example, methylcellulose and psyllium can cause bloating and flatulence secondary to their fermentation. Existing clinical trial data of such medications against placebo or other laxatives is scarce. Other examples of these agents are bran, calcium polycarbophil, and stool softeners such as docusate sodium and liquid paraffin, which can be effective in assisting stool evacuation and can be given as an enema.

2. *Stimulant laxatives* can induce intestinal secretion and propulsive movement and also stimulate sensory nerve endings in severely constipated patients including those with slow colonic transit (Kamm et al., 1992b). A recent review highlighted the limited evidence base supporting their use and also indicated that they can interfere with electrolyte balance within the gut (Emmanuel, 2011), however; they are considered the first line laxative option in treating STC (Wald, 2002). One of the common side effects of its chronic use is melanosis coli (Freeman, 2008). Pharmacologically, these can be subdivided into 3 main groups: anthranoids (e.g. senna); polyphenol derivatives (e.g. phenolphthalein, bisacodyl and sodium picosulphate); miscellaneous (e.g. docusate sodium, which is also a stool softener and bulking agent).

3. *Osmotic laxatives* such as sorbitol, mannitol, lactulose, magnesium, polyethylene glycol (PEG) salts etc. These increase fluid content of the stool by inducing water retention in the gut lumen, which facilitate stool expulsion. They are usually prescribed to treat both chronic and intermittent constipation. Their side effects are electrolyte imbalance, diarrhoea, abdominal bloating, nausea, and flatulence. A study involving only 8 patients with STC, showed improvement in their reported symptoms and a decrease in transit time from 91 h to 43 h (using ROMs) after the use of PEG 4000 (Klauser et al., 1995). Similar results have been reported by Bassotti *et al* using a similar agent but at a low dose (Bassotti et al., 1999b). However their use in general in STC patients is limited with only a minority of patients reporting improvement (Wald, 2002).

B. Biofeedback (behaviour therapy)

This is also called "neuromuscular training", and is an instrument-based treatment used to restore normal defaecation behaviour. This technique can either use visual, sensory, or auditory feedback during simulated evacuation to train new defaecatory behaviour (Rao, 2008). However, biofeedback is just one component of a 'bowel retraining' package. Advice regarding altering lifestyle, such as eating and drinking is also included. Although biofeedback has been used primarily to treat faecal incontinence (Engel et al., 1974), it has also proven beneficial in patients suffering from dyssynergic defaecation and obstructive defaecation (Rao, 2008), which can coexist in with STC (Zarate et al., 2009). Using EMG, anorectal manometry, or rectal balloon expulsion (to simulate passage of stool), there are two main objectives to treatment success, based on the underlying pathophysiology: (a) to correct the dyssynergia or incoordination of the abdominal, rectal, puborectalis and anal sphincter muscles in order to achieve a normal and complete evacuation; (b) to enhance rectal sensory perception in patients with impaired rectal sensation.

However, the mechanism underlying the changes in bowel function induced by biofeedback is still unclear. Thus the technique has been used to treat STC patients with evidence of co-existent dyssynergic/ obstructed defaecation. There are no randomised, controlled trials supporting its use in isolated STC. The evidence that biofeedback normalises colonic transit is conflicting; some studies report not only normalisation of measured transit time, but also improvement in reported symptoms

(Chiotakakou-Faliakou et al., 1998, Emmanuel and Kamm, 2001, Battaglia et al., 2004). In general, the rate of success of biofeedback is reported to be 20% - 100% (Chiotakakou-Faliakou et al., 1998, Brown et al., 2001, Wang et al., 2003, Battaglia et al., 2004). However, the success rate is greatly dependent on the protocol used during clinical practice, period of follow-up, degree of experience of the practitioner, patient motivation and convenience, and financial aspect. The duration and frequency of biofeedback training is based on an individual patient's needs. A single session can take up to an hour and can be performed twice a week and up to 6 total sessions for complete therapy (Rao, 2008).

C. Psychotherapy

Psychotherapy (e.g. hypnotherapy) has been used to treat constipation associated with irritable bowel syndrome (normal-transit constipation) (Webb et al., 2007, Spiller et al., 2007, Palsson and Whitehead, 2013). However, evidence for treating other types of constipation including STC is scarce.

D. Surgical

Surgical management may be offered to a small minority of patients who fail all available medical management and for whom the symptoms of constipation have a significant impact on their life style. Sir Arbuthnot Lane first described colectomy for refractory constipation 100 years ago (Lane, 1908, Lane, 1909). Other surgical procedures can also be performed. Examples of surgical options are antegrade colonic enemas, subtotal colectomy with ileorectal or ceacorectal anastomosis, segmental colectomy, ileal pouch anal anastomosis, and creation of a stoma (Frattini and Nogueras, 2008). However, details of these procedures are beyond the scope of this thesis.

A review published by Knowles *et al* for surgical outcomes in STC, showed a median satisfaction and success rate of 86%, but median reoperative rate at 14% (Knowles et al., 1999a). The review also highlighted that the success rate was dependent on the type of resection performed, with a better rate reported for subtotal colectomy with ileorectal anastomosis than others (Knowles et al., 1999a).

More recently, surgically implanted devices have been introduced as an option to treat STC by neuromodulation. Based on prolonged pancolonic manometric studies, sacral nerve stimulation (SNS) has been shown to induce colonic propagating

activity in STC patients (Dinning et al., 2007, Dinning et al., 2012). In a prospective study carried out in five European centres, SNS treatment resulted in improvement in symptoms of evacuatory dysfunction, abdominal pain and bloating, and bowel movement frequency (in patients with both slow and normal transit constipation (Kamm et al., 2010).

1.5.6.2. Novel therapies

Several new agents have recently become available to treat chronic constipation. These include 5-HT_4 serotonin receptor agonists, pharmabiotics (probiotics), and intestinal secretagogues.

Two 5-HT_4 serotonin receptor agonists (Cisapride and Tegaserod) have been withdrawn from the market due to potential serious adverse cardiovascular events. However, another 5-HT_4 receptor agonist (Prucalopride) is now available to treat women suffering from chronic constipation and for whom laxatives failed to alleviate their symptoms (Quigley, 2012). This acts by increasing gut contractility and peristaltic reflexes, as well as modulating visceral sensitivity (Emmanuel et al., 2002).

Secretagogues act by increasing intestinal fluid secretion; an example is lubiprostone, which has recently been approved in the USA and in Switzerland for the treatment of chronic idiopathic constipation and IBS- constipation (Chamberlain and Rao, 2012). Recently, another secretagogue linaclotide (guanylate cyclase- C receptor agonist (GCCA)) has been also licensed for treating adult patients suffering from IBS- constipation (Rao et al., 2012, Quigley et al., 2013, Rao and Weber, 2014); however 5% of patients who used this drug withdrew due to the adverse event of diarrhoea.

Recent studies showed that in patients with IBS- constipation has a decreased concentration of secretory unconjugated bile acid, nonsecretory bile acid, and chenodeoxycholic acid, compared with healthy controls and patients with IBS-diarrhea (Shin et al., 2013, Wong et al., 2012). Elobixibat is a highly selective bile acid transporter that is able to modulate enterohelaptic bile acid circulation and increase bile acid synthesis resulting in increased bile acids concentration and within ileum. This drug has been shown to enhance colonic motility (accelerate colonic

transit) (Wong et al., 2011), induce looser stool consistency, and improve constipation symptom severity compared with placebo (Shin et al., 2013).

Probiotics are live microorganisms that when administered in adequate amounts confer a health benefit to the host. Probiotics have been proposed for the treatment of patients with constipation as they can modify the gastrointestinal microbiota, which are known to be altered in constipation (Zoppi et al., 1998, Khalif et al., 2005). They may also alter intestinal sensation (Ait-Belgnaoui et al., 2006, Rousseaux et al., 2007) and modulate motility (Quigley, 2007). Other studies showed that probiotics increase the production of lactate and short-chain fatty acids resulting in reduction in luminal pH, which is believed to enhance colonic motility and accelerate whole gut transit time (Salminen and Salminen, 1997, Waller et al., 2011). However, a recent meta-analysis showed that although probiotics accelerate whole and regional colonic transit, improvement in stool frequency and consistency, effect is dependent on the species of microbiota administered (Dimidi et al., 2014). Further clinical trials are required to determine which bacterial strains, doses, and duration of probiotics are most effective in treating constipation (Dimidi et al., 2014).

1.5.7. COLONIC PATHOPHYSIOLOGY OF STC

Colorectal sensorimotor and biomechanical dysfunction are widely accepted as the principal pathophysiological mechanisms underlying symptoms of chronic idiopathic constipation (including STC). These dysfunctions may exist within one or more colorectal region. Nevertheless, our understanding remains rudimentary at best and a better understanding of pathophysiology (molecular, cellular, neuronal, muscular, and functional) is essential to guide clinical management. Problems include:

1. inaccessibility of most colonic regions, which had resulted in our incomplete understanding of pancolonic sensorimotor and biomechanical functions in both health and disease. Most existing knowledge is derived from studies performed on distal parts of the colon only.

2. standardisation of physiological tests used to assess colonic function is still poor, making study comparison very challenging.

3. sensorimotor and biomechanical dysfunctions may occur in combination or in isolation, and may coexist with other identifiable causes of constipation such as structural abnormalities; this makes determination of their individual role in symptom development difficult.

The defining feature of STC is prolonged colonic transit time, which is by itself an indirect measure of colonic motility as described previously.

The major body of this thesis will concentrate on large bowel (colorectal) motility in the human (both in health and in slow transit constipation).

1.6. COLONIC MOTILITY IN HEALTH AND STC

Colonic motility is a term used to describe spontaneous and active movements within the large intestine. These include contraction and relaxation of the intestinal wall leading to various mixing and propulsive waves that travel along the alimentary canal. These movements are responsible for the transit, digestion and absorption of intraluminal contents. There are various activity patterns of colonic motility which include:

1. propulsion (also termed peristalsis) responsible for moving intraluminal content in one direction (usually the aborad direction). This occurs as a result of synchronisation of smooth muscles within the gut wall

2. mixing patterns resulting from muscular contraction and relaxation that induces mixing of intraluminal food content with intestinal secretion, and also maximising its contact area with the intestinal wall to optimise food digestion and nutrient absorption. Both contraction patterns are reflected by changes in intraluminal pressure.

1.6.1. MEASUREMENT AND INVESTIGATION TECHNIQUES FOR ASSESSING COLONIC MOTILITY

Colonic motility encompasses one or more of four separate components: myoelectric activity; phasic contractile activity; tonic contractile activity; and movement of intraluminal content (transit) (Scott, 2003). Each component requires specific methods for assessment and no single test can provide assessment of all four activities simultaneously (Scott, 2003). Our knowledge of normal and abnormal colonic motility and mechanisms governing these motor functions remains incomplete. Historically, this has been due to the relative inaccessibility of this organ. Nevertheless, there have been many recent advances in the development of techniques to increase our knowledge of gastrointestinal motility and specifically colonic motility; some of these techniques are available for clinical use, while others remain limited to specialist research centres. The following section summarise the measurement of colonic motility recorded *in vivo* (human studies).

1.6.1.1. Myoelectrical colonic activity

Historically, studying colonic electrical activity was more complex than studying upper GI tract activity. Most of the knowledge of colonic myoelectrical activity or 'basic electrical rhythm', had been obtained from *in vitro* animal studies of colonic smooth muscle. *In vivo* human studies have been limited mainly to the rectosigmoid segment (Taylor et al., 1974). Traditionally, an electromyography (EMG) technique has been used to record colonic myoelectrical activity; however, it is now rarely used in practice (Scott, 2003). It can be performed using tube-mounted electrodes available in three types: serosal, intraluminal, or subcutaneous (Taylor et al., 1975) each of these are believed to measure different electrical activities. The first two types of electrodes need to be introduced by endoscopic assistance, while the cutaneous type is directly attached to the abdominal wall. The technique is relatively inexpensive and easy to perform. However, many external and internal factors within the colon can induce change in electrical activities and artefacts are also commonly reported (Sarna, 1991). Early human *in vivo* studies showed that the basic electrical rhythm is present in a form of 'slow wave', which occur as intermittent sequences (Couturier et al., 1969). In 1974, Taylor and his colleagues described rectosigmoid activity in man, using mucosal suction electrodes introduced by sigmoidoscope assistance; they showed two main rhythms at rest: fast rhythm (frequency 6 - 10 cycles/min), and slow rhythm (frequency 2.5 - 4 cycles/min) which had a higher amplitude. They also reported that these waves were more predominant in the distal rectum than the upper rectosigmoid (Taylor et al., 1974) (Figure 1.05). The group further studied proximal colonic activity in 1975, using intubation via stomas, with additional subcutaneous electrodes (Taylor et al., 1975). They observed that the low frequency rhythm exists in a similar frequency at all levels of the large bowel, while the high frequency rhythm when present, varies along the whole length of the colon and occurs more commonly in the proximal colon than rectosigmoid. Similar frequency ranges were later described by others (Snape et al., 1976, Bueno et al., 1980, Sarna et al., 1980, Sarna et al., 1981). The correlation between colonic electrical activity and phasic contractions is an area of much controversy (Ritchie et al., 1962, Sarna et al., 1982, Frieri et al., 1983, Wingate and Kumar, 1992).

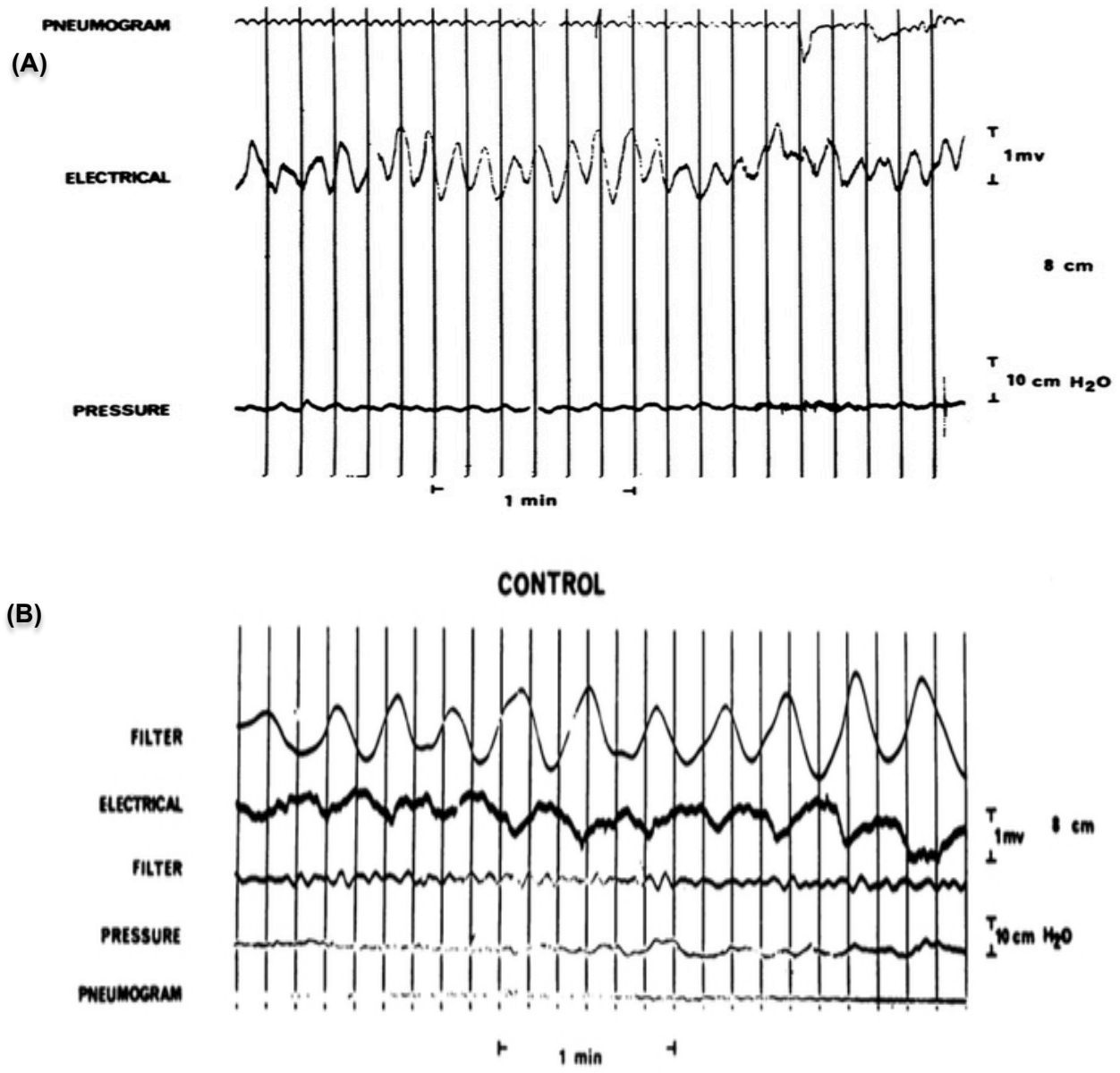


Figure 1.05. Recording of basic rectosigmoid electrical activity in human showing **(a)** fast electrical activity and **(b)** slow electrical activity. (Recording adopted from (Taylor et al., 1974)).

1.6.1.2. Colonic transit

Various methods have been used in an attempt to measure colonic transit, including the use of radiological imaging (with bismuth or barium sulphate meal as a marker) (Hurst, 1919, Barclay, 1936), particulate matter (such as glass beads along with an insoluble powder) (Alvarez, 1924), chemicals (such as copper or barium) (Alvarez, 1924), and isotopic materials (such as ^{51}Cr-labelled sodium chromate (Hinton et al., 1969). The physical properties of these different markers such as weight and specific gravity differ, which could itself affect gut motility.

Presently, two main methods are available to measure whole gut and colonic transit, and both involve exposure to ionising radiation: the ingestion of radio-opaque markers followed by plain abdominal x-rays, or suitable *radionuclide* markers with progression followed using a gamma camera (colonic scintigraphy).

1.6.1.2.1. Radio-opaque marker studies (ROMs)

This method was first described by Hinton *et al* in 1969. Subjects ingested radio-opaque, cylindrical polythene pellets, and their disappearance from the gut was measured by their appearance in stool using successive radiographic images (Hinton et al., 1969). This study determined that in males, 80% of the markers were expelled by 5 days. Subsequently, normal ranges for both genders have been established, and the retention of more than 20% of markers on a single plain radiograph performed at day 5 following ingestion has been defined as delayed colonic transit (Figure 1.06) (Bassotti et al., 1988, Evans et al., 1992, Hinton et al., 1969). However, this method does not provide information on segmental transit. Consequently, many protocols have been developed, involving the administration of single or multiple sets of radio-opaque markers, that require single or multiple radiographic images in order to extend the use of this technique to identify regional gut and colonic transit times. There is great variation in methodology regarding this technique [10 published methods involve ingestion of a single set of markers, and at least 5 methods involving ingestion of multiple sets of markers (Dinning et al., 2009a)].

In most studies, it is actually whole gut transit rather than colonic transit that is being measured. Only a reliable colonic delivery system (enteric coated capsules ensuring release of ROM upon entry to colon rather than in the stomach and small intestine) truly allows study of colonic transit.

Table (1.02) summarises validated methods for administration of the ROM. Normative data for colonic transit times have been established in healthy volunteers from many studies (Metcalf et al., 1987, Chaussade et al., 1989, Sadik et al., 2003, Southwell et al., 2009). The average time for colonic transit in health is reported to be between 30 and 70 h. Gender differences in colonic transit time using ROM methods are also reported with females shown to have longer colonic transit time (Southwell et al., 2009). However, differences in absolute colonic transit times published thus for very likely differences in methodology and the lack of universal standardisation of this technique.

Three main patterns of transit delay in constipated patients have been reported using ROMs: (a) generalised, when markers are distributed in all colonic regions (also called colonic inertia); (b) left-sided delay, when the majority of markers are located between the distal transverse colon and the rectosigmoid region; (c) rectosigmoid delay, when the majority of markers are located within the sigmoid and rectum (Metcalf et al., 1987).

From several published studies, the mean diagnostic yield of radio-opaque marker studies in identifying delayed colonic transit in patients with chronic constipation is 44% (range 13 – 68%) [data from 12 studies, with >30 subjects recruited] (Dinning et al., 2009a). However, the clinical utility of ROMs in constipated patients remain debated (van der Sijp et al., 1993, Dinning et al., 2009a)

The main advantages of this technique are simplicity to perform in daily clinical practice, relatively inexpensive, and universal availability. However, the test obviously involves irradiation of the subject under study. It also involves ingestion of non-physiological markers, which may not move throughout the bowel in a similar manner to intestinal content (Krevsky et al., 1986). Furthermore, some patients show poor compliance in ingesting ROM at specific times, notably on multiple occasions, which can interfere with interpretation of study results. Intracolonic localisation of ROM can also sometimes be difficult with limited anatomical landmarks, overlapping

bowel segments, and also an impacted colon, where stools can reduce the visibility of ROMs on a plain abdominal radiograph (Krevsky et al., 1986).

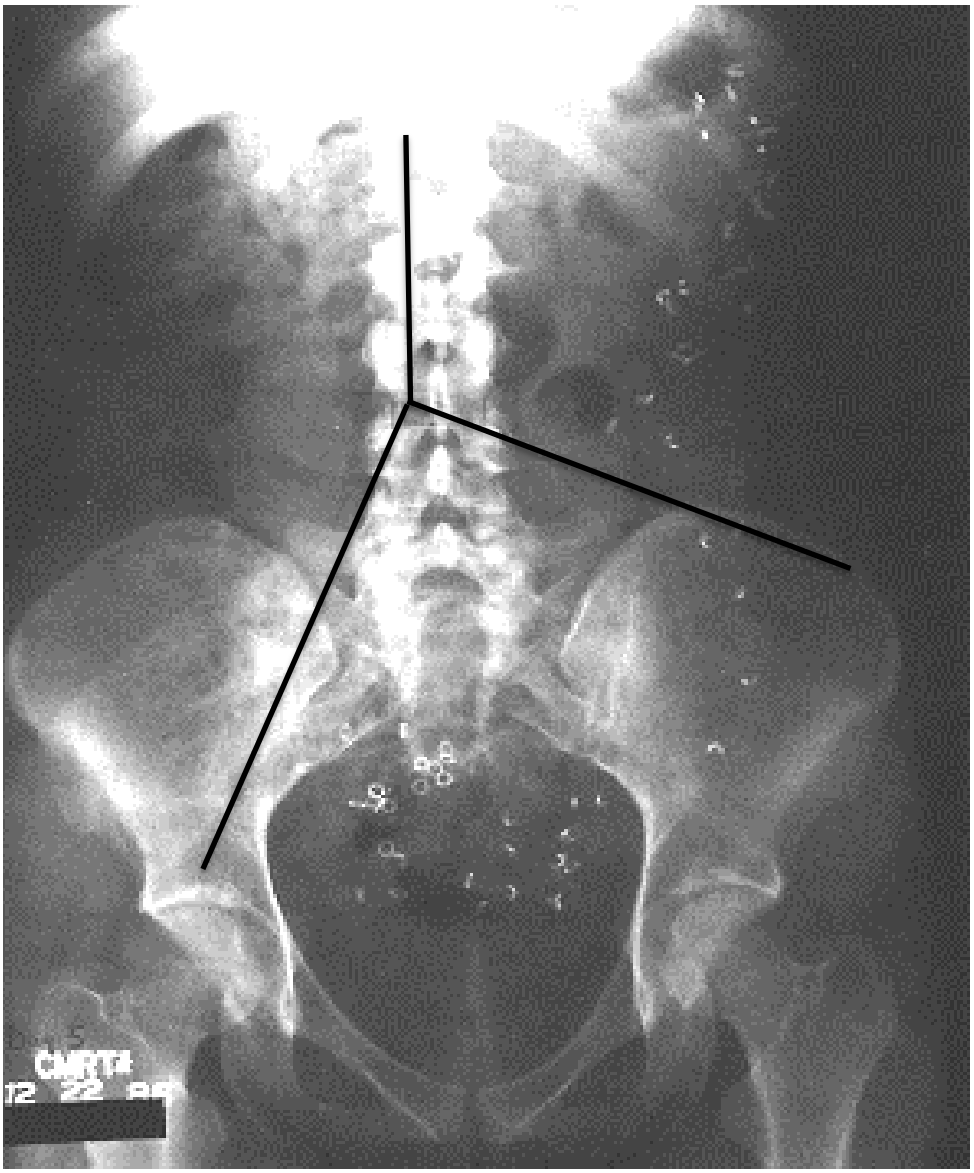


Figure 1.06. Radio-opaque marker study (ROM). An anterior abdominal x-ray 100 h following ingestion of radio-opaque markers (ring shape), which are retained mostly on the left side of the colon. Lines are usually drawn to divide the abdominal radiograph into three main regions based on known anatomical landmarks: right and left colon, and rectosigmoid area; this can help with marker localisation.

Table 1.02. Validated methods of administration of radio-opaque markers (ROMs). TT: transit time, MTT: mean transit time, HV: healthy volunteers, WGT: whole gut transit, RCT: regional colonic transit, WCT: whole colonic transit, CTT: colonic transit time, M: male, F: female.

Studies	Year of study	Total number of markers	Total number of x-rays	Time of x-rays	Gut region studied	Study population	Findings
(Hinton et al., 1969)	1969	20	Serial	3rd day, 5th day	WGT	25 HV (M)	All subjects pass their first marker within 3 days and 80% of ROM within 5 days
(Cummings and Wiggins, 1976)	1976	20	1	4th day	WGT	22 HV (M)	The average MTT was 54.2 h ± 2.6 h obtained from single x-ray and reported to be more accurate and reproducible method than the 80 % TT.
(Arhan et al., 1981)	1981	20	3	1st day, 4th day, 7th day	WCT, RCT	Adult and children	MTT in *right colon 13.8 h (adult) vs. 7.7 h (paediatric) *left colon 14.1h (adult vs. 8.7h (paediatric) *rectum 11h (adult) vs. 12.4h (paediatric)
(Metcalf et al., 1987)	1987	3 set of 3 different shapes of ROMs (20 each) given over 3 successive days	1	4th day, 7th day	WGT, WCT, RCT	73 HV (F+M)	*CTT= 35.5+/-2.1h *right CTT=11.3 ± 1.4 h *rectosigmoid transit= 12.4 ± 1.1h
(Abrahamsson et al., 1988)	1988	6 sets of ROMs (10 each) given over 6 days, additional 20 markers given on day 6)	1	7th day	WGT, WCT, RCT	56 HV (F+M)	WGT in female (2.4) days vs. (1.9) days in male
(Chaussade et al., 1989)	1989	3 sets of identical ROMs (20 each) given over 3 successive days.	2 -3	4th day, 7th day, Additional x-ray at day 10 if needed	WCT, RCT	91 constipated patients	Able to sub-classify patients into normal colonic transit, right and left colonic stasis, and outlet obstruction
(Evans et al., 1992)	1992	single set of marker (no?) given in one occasion	2	12 h and 120 h post ingestion	WGT	43 HV (F+M)	Normal subjects retain more than 20% of markers within 12 h and less than 80% after 120 h

1.6.1.2.2. Isotope transit studies

Whole gut and colonic transit times can be measured by ingestion of a suitable gamma-emitting radionuclide (radioisotope) and following its progression through the gut using a gamma detection camera. This technique is known as whole gut and colonic scintigraphy respectively.

Historically, the first isotope study to investigate intestinal transit was performed in 1962, and used ^{51} Chromium [^{51}Cr]-labelled sodium chromate (in a liquid form for oral administration) (Hansky and Connell, 1962). ^{51} Chromium is poorly absorbed in the GI tract but its half-life is long (approximately 26.5 days). This was followed by attempts to use other isotopes with shorter half-lives and also to specifically measure colonic rather than whole gut transit. Different delivery methods of the radioactive materials have been published. In 1986, colonic transit time was specifically studied using oral intubation of the caecum in seven healthy volunteers with the administration of ^{111}indium bound to diethylenetriamine pentaacetic acid [^{111}In DTPA] (Krevsky et al., 1986); this is poorly absorbed throughout the gut with a half life of approximately 67 - 77 hours, which should be enough to perform prolonged observation of colonic transit. A similar technique using ^{111}In was also described by Kamm *et al* in 1988 (Kamm et al., 1988); this required colonic placement of a 2 mm tube passed orally and multiple fluoroscopic scans to follow its progression to the desire location. Thereafter, McLean *et al* proposed the use of Iodine-^{131} cellulose as an alternative isotope (followed by multiple scans to follow its progression for up 96 hour) (McLean et al., 1990); this isotope was clearly able to identify delayed colonic transit (mean total percent of colonic retention of Iodine was 48% in HV and 84% in constipation at 24 h, 11% in HV and 63% in constipation at 48 h, and 3% in HV and 43% in constipation at 72 h) (McLean et al., 1990). However, the preparation of this isotope is time consuming and also its half-life time is longer than that of ^{111}In DTPA, which exposes patients with delayed colonic transit to a higher radiation dose (McLean et al., 1990). This led to the development of an oral preparation of ^{111}In [DTPA]. Smart et al used both ^{111}In [DTPA] and I^{131} Cellulose and obtained serial images obtained at 6, 24, 48, 72, and 96 h). They concluded that patterns of colonic transit obtained using both isotopes were identical. However, ^{111}In [DTPA] images were of better resolution than I^{131} Cellulose (Smart et al., 1991). The technique of the administration of ^{111}In [DTPA] has subsequently been developed further for more

practical clinical use (McLean et al., 1992, Maurer et al., 1992). Other delivery methods of ^{111}In [DTPA] have also been described in the literatures, such as time-release capsules filled with ^{111} In (Stivland et al., 1991, Notghi et al., 1994, Charles et al., 1995), or using activated charcoal mixed with ^{111}In, stored in a pH-sensitive methacrylate-coated capsule that dissolves and releases the isotope within the alkaline pH of the distal ileum (Burton et al., 1997).

Colonic scintigraphy is now a well-established technique in clinical practice; two methods are commonly used though the numbers of centres employing these methods is very limited: (i) oral administration of ^{111}I [DTPA], with scans taken once or twice per day up to 72 or 96 h (McLean et al., 1992, Roberts et al., 1993) and (ii) ^{111}In delivered via methacrylate-coated capsules with scans taken at 4, 24, and 48 h (Burton et al., 1997). In health, the colon normally empties in a near linear manner after a lag phase (secondary to colonic storage). McLean *et al* studied the variability of colonic transit in healthy volunteers, and showed that there was small but significant difference in colonic transit time between females and males (colonic transit time longer in females than males), which should be always considered when performing data analysis (McLean et al., 1992).

In term of data acquisition and analysis, various methods have been described (Krevsky et al., 1986, Roberts et al., 1993, Scott et al., 2001). Briefly, a computer-based image of the colon is developed based on images obtained during scintigraphy. The colon is then divided into seven regions (regions of interest: ROI) (1 is the caecum and ascending colon, and 6 is the region of sigmoid colon and rectum; region 7 represents expelled faeces) (Figure 1.07). Other protocols, particularly those adopted by the Mayo Clinic, divide the colon into five rather than seven ROIs (Camilleri and Zinsmeister, 1992, Cremonini et al., 2002). In each ROI, the percentage of radioactive material is calculated and then time-activity curves can be created and represent the progression of the isotope's geometric centre (GCI) over the study period. A low value for geometric centre (toward 1) implies that the majority of the radionuclide marker is in the caecum and ascending colon, whereas a high geometric centre value indicates that the majority has been expelled (Dinning et al., 2009a). Additional parameters can also be measured to indicate the severity in transit delay, such as gradient of GCI progression and estimated evacuation time (Scott et al., 2001). Various patterns of colonic transit delay have been reported in

STC patients. However, it is not clear whether these patterns can influence management. Three subtypes can generally be identified: (1) a generalised delay (Maurer and Krevsky, 1995); (2) right-sided or left-sided hold-up, (3) functional rectosigmoid obstruction, which is believed to be associated with functional outlet obstruction (Krevsky et al., 1986, Roberts et al., 1993, Maurer and Parkman, 2006). Whether the latter represents a primary delay within the very distal colon, or is secondary to a co-existent evacuatory problem is still unclear (Dinning et al., 2009a). The principal site of hold-up remains debated. Krevsky *et al*. and Stivland *et al*. indicate that the delay was in the right colon in most constipated patients (Krevsky et al., 1989, Stivland et al., 1991), whereas Roberts *et al*. showed that the delay is mainly present in the transverse colon and at the splenic flexure (Roberts et al., 1993). Zarate *et al* reported no difference in transit delay (based on geometric centre of isotope progression) between those with isolated STC, and patients suffering from STC and RED (as diagnosed by a preceding radio-opaque marker screening test and evacuation proctography, respectively) (Figure 1.08) (Zarate et al., 2008). It may be that current imaging protocols are inadequate to fully identify more subtle patterns of colonic transit delay. Furthermore, assessment of transit by scintigraphy is usually only performed in those who have already been found to have a transit delay on a screening radio-opaque marker study. Whether some patients have a segmental transit delay in the presence of overall normal transit time is also unclear. Thus there is a need to further develop clinically useful diagnostic criteria using scintigraphy to better delineate patients with constipation into more homogenous groups based on their colonic transit pattern. This is certainly true for male patients with constipation, in that STC, based on standard measures of colonic transit, is believed to be an almost exclusively female disorder (Knowles et al., 2003).

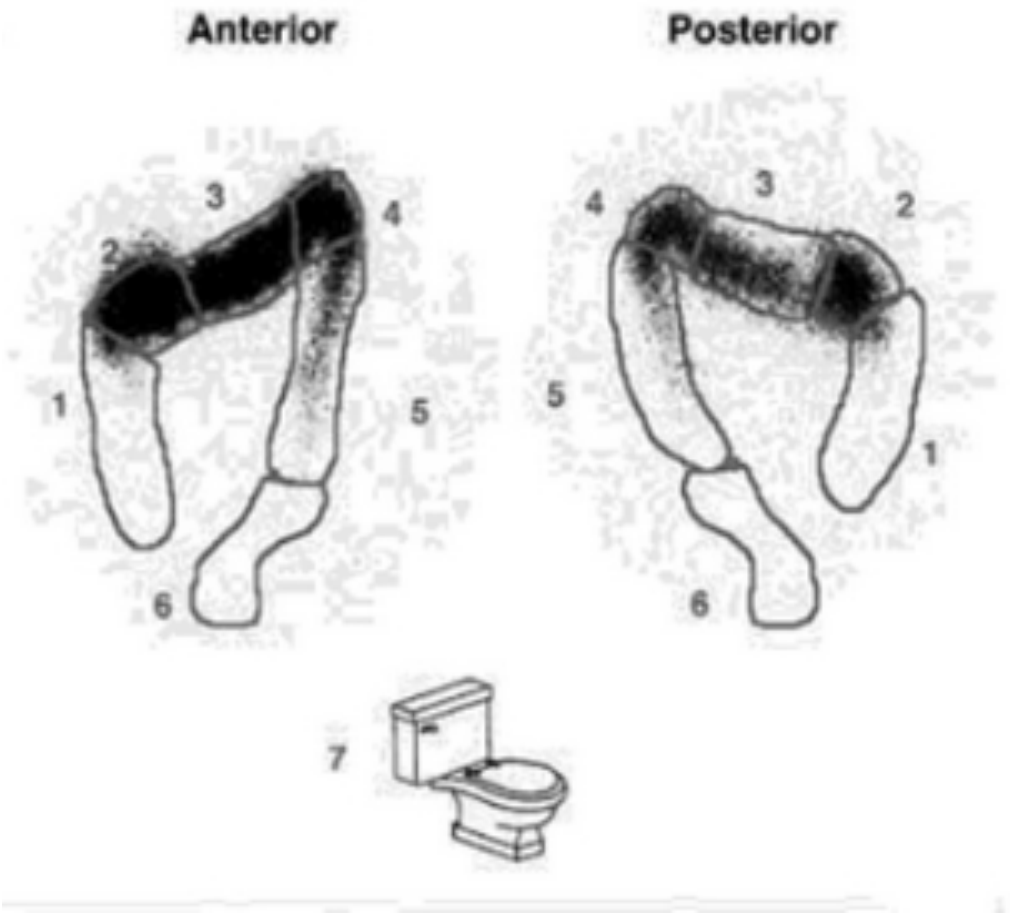


Figure 1.07. Calculation of colonic transit using the geometric centre technique. Regions of interest (ROI) are generated around six colonic segments on both anterior and posterior images. The activity count in ROI 7 is equal to the difference between input activity in the colon and the total activity count in the colon. Figure adopted from Baert (Baert, 2006).

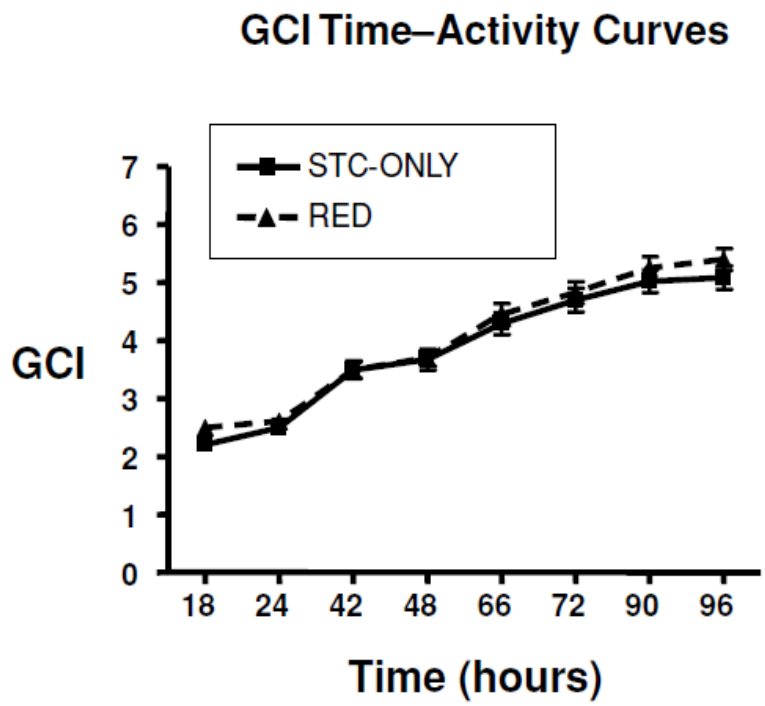


Figure 1.08. Time-activity curve: the geometric centre of isotope mass (GCI). Y-axis represents GCI value progression over time (X-axis). In this example patients with isolated slow transit constipation (STC-only) and those with coexistent rectal evacuatory dysfunction (RED) had equivalent GCI time-activity curves (Zarate et al., 2008).

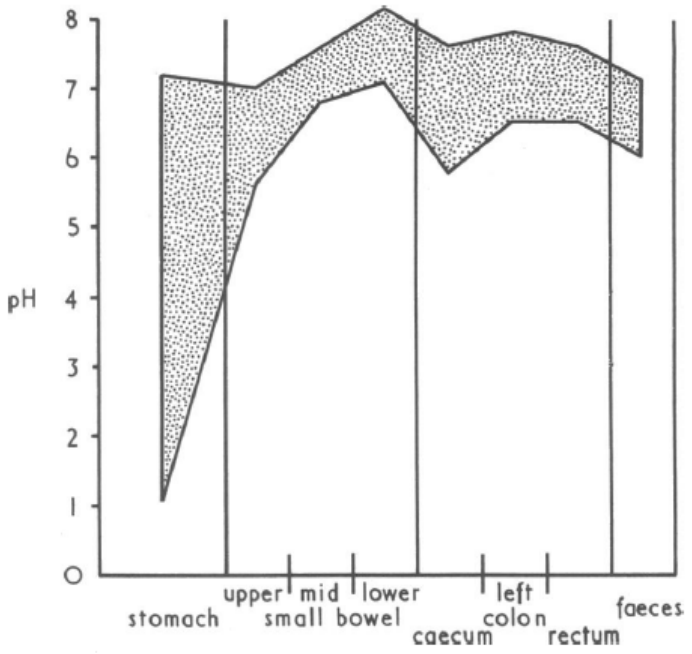
Isotope scintigraphy is recognised as the 'gold standard' for measuring colonic transit time, as it provides more accurate and more easily quantifiable information than radio-opaque marker studies (Lin et al., 2005). Scintigraphy also provides a better assessment of regional colonic transit than radio-opaque markers, and can be used in combination with a labelled meal to assess gastric emptying and regional gut transit (Bonapace et al., 2000). However, overall colonic transit times as measured by ROM and scintigraphic methods show that ROM slightly move faster than the centre of mass (van der Sijp et al., 1993).

The main limitations of scintigraphy are the use of radiation (albeit less than with radio-opaque markers) and by relatively long study duration (as the subject needs to attend the study centre multiple times for multiple scans to follow the movement of the isotope throughout the colon for up to 7 days). Furthermore, it is relatively expensive, compared to the ROM studies.

1.6.1.2.3. Telemetric devices

The concept of ingestible telemetric capsules incorporating sensors for studying GI functions started over 50 years ago (Jacobson and Mackay, 1957, Farrar et al., 1957); Connell constructed a pressure-sensitive radio-pill that was able to measure colonic pressure changes (Connell et al., 1963). In 1972, a pH-sensitive radiotelemetric capsule was used to describe changes in the pH profile along the GI tract, including the colon (Watson et al., 1972). Stereotypical changes in pH occur throughout the intestinal lumen: after ingestion there is an immediate fall in pH as the capsule enters the acidic environment of the stomach. This is followed by a sharp rise on exiting the stomach and a further fall in pH some hours later as the capsule is believed to pass from the ileum into the colon (Figure 1.09 A). More than a decade later, the pH profile of the gut was described in 66 healthy controls (Evans et al., 1988). A sharp fall in pH at the point where the radio-pill is assumed to pass from the terminal ileum to the caecum was reported. This allowed the authors to present regional gut transit times based on these pH 'landmarks' (Figure 1.09 B).

(A)



(B)

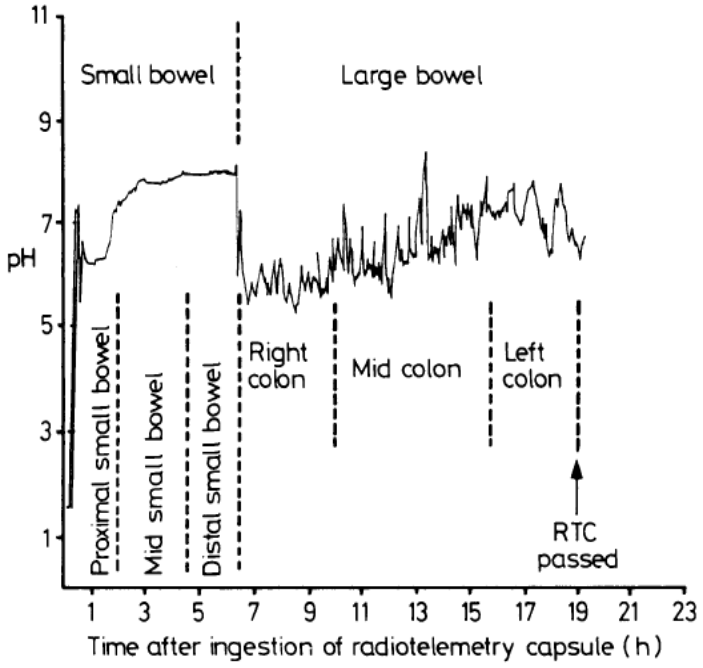


Figure 1.09. pH profile throughout the gut. (**A**) The first description of pH profile within the gut was described by Watson *et al* (Watson et al., 1972). The shaded area represents the extreme value of pH profile observed in 9 subjects (2 healthy control and 7 patients with various gastrointestinal diseases). (**B**) Recorded pH profile from healthy subjects as described by Evan *et al* (Evans et al., 1988). The assumed location of the capsule is highlighted in each intestinal segment based on pH profile and the time from capsule ingestion.

The exact location of this fall in pH around the ileocaecal junction (ICJ) has, however, never been conclusively substantiated. If pH-sensitive devices are to be used to measure gut transit times, it is fundamental to the validity of such a technique that the precise anatomical location of any pH change is verified. Therefore, reproducibility and further clinical validation of such technology for assessing regional gut and colonic transit are still required.

Pressure-sensitive radio-pills have also been used to assess colonic motility (Thorburn et al., 1992). Recently, new commercially produced devices have become available for the measurement of colonic transit and contractility (Camilleri et al., 2008). These include the wireless motility capsule (SmartPill; SmartPill Corporation; USA) and the magnet tracking system (Magnetic Pill; Motilis Medica SA; Switzerland).

Further detail of the wireless motility capsule (SmartPill) is described in Chapter 6 and 7.

1.6.1.3.4. Factors influencing colonic transit time

1.6.1.3.4.1.Gender

Differences in colonic transit time between genders have been reported in a large number of studies (mainly ROM methods) (Stephen et al., 1986, Metcalf et al., 1987, Abrahamsson et al., 1988, Lampe et al., 1993, Meier et al., 1995, Sadik et al., 2003, McLean et al., 1992, Graff et al., 2001, Madsen et al., 2003, Degen and Phillips, 1996). All of these studies, regardless of technique, confirm that colonic transit time is significantly faster in men than women ($P< 0.05$). Nevertheless, a few studies have failed to report any gender differences (Madsen, 1992, Evans et al., 1998). Female sex hormones, mainly progesterone, have been suggested as an important reason for such differences; however, their effect is still not clearly understood. Animal studies have suggested that female sex hormones actually decrease the contractility of colonic smooth muscle (Bruce and Behsudi, 1980, Ryan and Bhojwani, 1986, Chen et al., 1999). Furthermore, changes in colonic transit times in healthy women during different phases of the menstrual cycle and during pregnancy are inconsistent (Wald et al., 1981, Lawson et al., 1985, Hinds et al., 1989, Meier et al., 1995). Recently, Gonenne *et al* examined the effect of sex hormone supplementation and withdrawal on postmenopausal women; they concluded that

progesterone does not appear to have any effect on colonic transit time as measured using a scintigraphic method (Gonenne et al., 2006).

1.6.1.3.4.2. Ageing

The effect of ageing on colonic transit has been widely investigated, the majority of studies show no correlation between age and increasing transit times (Metcalf et al., 1987, Merkel et al., 1993, Meier et al., 1995), though Metcalf *et al* noted a tendency towards a longer mean colonic transit time in older subjects (Metcalf et al., 1987). Other studies have also reported slower colonic transit in older compared to young healthy subjects (McLean et al., 1992, Madsen et al., 2003). Madsen and Graf also reported that normal ageing appears to reduce the propulsive capacity of the colon based on colonic transit times measured by colonic scintigraphy (Madsen and Graff, 2004). The effect of ageing on colonic motor activities (recorded directly) is not understood, with no *in vivo* physiological studies yet performed.

1.6.1.3.4.3. Body mass index

The effect of obesity, expressed as increased body mass index (BMI) (kg/m^2), on colonic transit time has also been examined in healthy subjects. Constipation and diarrhoea are often both reported in overweight and obese people. Serial studies performed by Madsen and colleagues showed no correlation between BMI and the progression of geometric centre using isotope scintigraphy (Madsen, 1992, Madsen et al., 2003, Madsen and Graff, 2004). Sadik *et al* also showed no effect of BMI on colonic transit using ROMs (Sadik et al., 2003). However, Delgado-Aros *et al* studied the effect of high BMI on colonic sensorimotor and transit functions in healthy controls (Delgado-Aros et al., 2008), and they reported an overall significant acceleration of colonic transit with BMI > 30 kg/m^2 (P = 0.003); however, this result was significantly influenced by gender (P value was only 0.08 when data adjusted by sex). In fact, no clear underlying pathophysiology has yet explained these changes. Various factors should be considered when examining the effect of obesity on colonic transit time, such as dietary habit, level of physical activity etc. that can all contribute to the speed of colonic transit.

1.6.1.3.4.4. Dietary fibre and fluid intake

Constipation has been attributed to the lack of dietary fibre, which usually is the first-line recommendation by most physicians, in addition to other lifestyle modifications, to improve simple and chronic constipation. The daily recommended fibre intake is 20 g to 35 g daily. There are two types of fibre: soluble (which can absorb water and form a gel-like material during digestion) and insoluble fibres (which can add bulk to intestinal content and facilitate its movement through the bowel). However, there are only a handful of good clinical trials that have examined the effect of fibre on constipation (Suares and Ford, 2011, Eswaran et al., 2013). A recent systemic review concluded that despite national and international guidelines recommending increasing fibre intake in constipation, the evidence for this is limited (Eswaran et al., 2013). The review also indicated that use of soluble fibre, such as psyllium, proved more beneficial in alleviating constipation symptoms and increasing frequency of bowel movement compared to insoluble fibre (Eswaran et al., 2013).

Inadequate fluid intake is also though to be a frequent causes of chronic constipation. Increasing daily fluid intake considered one of the initial lifestyle modifications to improve constipation symptoms. However, there are very limited studies questioning the effect of reduced fluid intake/ fluid deprivation on colonic motility (specifically on colonic transit time). One study which screened on elderly population (mean age of 74 years) indicated that reduce water intake had a moderate association with self-reported chronic constipation (Lindeman et al., 2000). Decreased fluid intake is reported to increase the risk of fecal impaction (Wrenn, 1989). Another study examined the effect of water deprivation for one week (< 500 ml/ per day) compared to a fluid intake of 2500 ml of fluid per day for a similar period on whole gut transit in a small group of healthy males (Klauser et al., 1990). This study showed a significant difference with regard to stool frequency and stool weight ($P < 0.04$) but no difference in gut transit time between the two weeks. Therefore, fluid intake may play a role in adjusting stool consistency, but there is no strong evidence available on its effect on altering colonic motility.

1.6.1.3.4.5. Physical activity

The impact of exercise and the level of physical activity on whole gut and colonic transit have been debated for a long time; results remain controversial. This is mainly due to methodological issues, such as the duration and the intensity level of exercise and the method of measuring colonic transit. There are a limited number of clinical trials which generally show no difference in transit time based on the level of physical activity in either patients with delayed colonic transit or healthy subjects (Cordain et al., 1986, Metcalf et al., 1987, Coenen et al., 1992, Robertson et al., 1993, Song et al., 2012). However, a few studies have shown a reduction in colonic transit time with exercise (Oettle, 1991, Koffler et al., 1992); nevertheless, these studies were carried out on small samples (maximum number of 10 healthy controls). De Schryver *et al* examined the effect of physical activities in 43 middle-age women suffering from constipation; they reported improvements in both symptom scores and a decrease in colonic transit time following a 12 week program of regular physical exercise (rectosigmoid transit time reduced from 17.5 h at baseline to 9.6 h, and total colonic transit time from 79 h to 58 h; $P < 0.05$) (De Schryver et al., 2005). A recent study also showed that women with a high level of physical activity have shorter right colonic (2.4 ± 4.4 h vs. 10.4 ± 10.6 h; $P = 0.004$) and recto-sigmoid (3.8 ± 7.0 h vs. 21.8 ± 14.4 h; $P = 0.02$) transit times (measured by ROMs) compared with those with low level of physical activity.

1.6.1.3. Colonic motor activity

The colon expresses two types of contractions: *phasic* and *tonic* contractions. Definition and details of each type are shown below.

1.6.1.3.1. Phasic colonic motor activities

Colonic phasic contractile activities appear to result from contractions of the colonic circular smooth muscles, rather than the longitudinal muscles of the colonic wall, and are expressed as changes in intracolonic pressure (Scott, 2003). These contractile activities have a pivotal role in the movement of intraluminal colonic content.

1.6.1.3.1.1. Historical data

The earliest observations of colonic movements were described by Bayliss and Starling, using direct observations of movement in denervated canine colons (Bayliss and Starling, 1900). However, such a technique is impractical for physiologically studying the human colon due to many confounders, including surgical intervention and tissue handling. This first major report describing colonic motility *in vivo* in the human was performed by Holzknecht in 1909. During this study, he followed the progression of a bismuth meal throughout the gut using more than one thousand radiographic images. He reported 'mass peristalsis' (described as a wave of colonic contraction starting from the proximal transverse colon and moving rapidly within seconds to the rectosigmoid area and leading to disappearance of interhaustral folds for short period). He concluded that colonic activity may be limited to infrequent sudden movement that occur probably 2 - 3 times per day (Holzknecht, 1909a).

In 1911, Schwarz attempted to repeat the observation of colonic movement using a similar technique and concluded that the shape and location of colonic haustrations were slowly changing most of the time and are more predominant in the proximal rather than the distal colon (Schwarz, 1911). This was followed by work performed by Hertz and Newton in 1913; they described rapid changes in haustrations located mainly within the caecum and ascending colon. In addition, they stated that colonic propulsive movement induced a tubular appearance of the colon (Hertz and Newton, 1913). They also observed that colonic contents (a bismuth meal) travelled further during the mealtime than during the fasting state (i.e. gastro-colonic response). Similar findings were also reported by Barclay in 1912 in terms of colonic movement

during a meal and also mass peristalsis (Barclay, 1912b). More than three decade later, Barclay also then described colonic content movement as slow and intermittent with no sign of directional intention (Barclay, 1935). Overall, these studies led to the colon being described as 'an inactive or still organ', as shown in many physiology textbooks in the early and mid-twentieth century (Herbert and Burke, 1990).

These primitive findings were succeeded by more focused research on colonic movement in the 1960's and 1970's, where Ritchie, Ardran, and Truelove used time-lapse cineradiography with and without intraluminal pressure recordings to more accurately record colonic movements (Ritchie et al., 1962, Ritchie et al., 1971, Ritchie et al., 1968). They described new patterns of human colonic movement that occur in 'slow flow' patterns, which were more frequent than mass movements. Furthermore, the authors reported that colonic movement could comprise both propulsive (mainly in an antegrade direction) and non-propulsive movements. Other studies in the mid part of the 29th century used various techniques to record intracolonic pressure changes, such as a relatively large intraluminal balloon filled with water or gas (Templeton and Lawson, 1931, Adler, 1941, Quigley, 1950, Spriggs et al., 1951) or miniature balloons connected to fine polyethylene open-ended intraluminal tubes filled with fluids attached to a metal capsule optical manometer (Connell, 1961, Chaudhary and Truelove, 1961a, Chaudhary and Truelove, 1961b, Chaudhary and Truelove, 1961c).

Later, colonic motility studies performed in the 1960s and up to the early 1980s broadly subdivided colonic contractility into two types: propulsive contractions (mass movements), and non-propulsive contractions (segmental activity); each type was reported to have a different function and to be effected by different types of muscle contraction, which may be mediated through separate pathways (Misiewicz, 1975, Trotman and Misiewicz, 1982). Such studies providing the cornerstone for further development of colonic manometric devices made from either multichannel water-perfused or solid-state catheters along with pressure-sensitive radiotelemetric sensors used for both human and animal *in vivo* studies.

In the late 1980's, the first prolonged colonic manometric study in healthy humans was performed by Narducci *et al* using long open-ended multi-lumen tubes perfused with fluid, incorporating side-openings spaced 12 cm apart. These were connected to

external pressure transducers. The catheters were introduced colonoscopically with prior bowel preparation to the transverse colon of 14 healthy subjects (Narducci et al., 1987). Colonic contractility was described as '*irregular, predominantly low-amplitude non-propulsive segmental contractions with period of quiescence, along with sporadic non-propagating contractions, non-propagating bursts of contractions, and high amplitude propagated contractions (HAPC) in the absence of regular cyclical motor activity'*. However, the effect of prior bowel preparation in not-ambulant subjects with continuous colonic perfusion was unclear. Furthermore, information from the ascending colon was missing.

The first attempt to record prolonged pancolonic motor activity in health under more physiological conditions was by Soffer *et al* in 1989, using per-nasal colonic intubation of a long catheter incorporating pressure sensors. This was connected to an external portable recorder in 9 fully ambulant healthy subjects with no prior bowel preparation (Soffer et al., 1989). The study showed that colonic contractions were *'sporadic and irregular, associated with infrequent bursts that did not follow any pattern; contractile activity throughout the large bowel was reduced to a minimum during sleep and enhanced on awakening and following meals'*.

Over a decade later, Dinning and Cook from Australia performed a seminal series of colonic manometric studies proving the feasibility of recording pancolonic motor activity using long water-perfused catheters with multiple pressure sensor that span the whole length of the large bowel (Bampton et al., 2000, Bampton et al., 2001, Dinning et al., 2008a). These studies described non propagating colonic pressure waves consisting of cyclic or individual activities to represent the major colonic motility pattern; such activities are likely to be associated with mixing and propulsion of colonic content (Dinning et al., 2008a). Additionally, antegrade propagating contractions were described, which have a close temporal relationship with luminal transit and increase prior to physiological stimuli (defaecation); this has led to an increased focus on such activities due to their evident physiological importance (Cook et al., 2000, Bampton et al., 2000, Dinning et al., 2008a). Dinning and Cook have also developed a unique space-time-pressure 'mapping' for prolonged colonic manometric recording that readily permits an overall view of colonic antegrade and retrograde colonic propagating patterns within a single figure (Dinning et al., 2008b).

This allows a better appreciation and identification of motility patterns in both healthy controls and constipated patients than previously feasible.

1.6.1.3.1.2. Colonic manometric technique

The technique of colonic manometry allows *direct* recording of colonic contractile activities over prolonged periods from the colon. In the context of hindgut motility, recordings in man have, to date, almost exclusively been limited to the recto-sigmoid region with few attempts to record pancolonic motility. In the majority of studies, catheter-mounted sensors have been positioned by colonoscopy, with the catheter tip placed within the left colon. Advancement of recording probes beyond the transverse colon has rarely been described using this method, and thus an appreciation of "pan-colonic" motility has not been obtained. Furthermore, colonic manometric procedures lack any standardisation in term of catheter type, placement techniques and protocols (the variability of catheter configuration, type and study protocol reviewed in detail by Scott 2003, and Dinning *et al* in 2009; summarised below in Table 1.03). Compared to other parts of the gastrointestinal tract, our understanding of colonic motility remains rudimentary at best. In fact only about 20 studies of true pancolonic motor activity exist in the medical literature (Scott, 2003, Dinning et al., 2010). Our knowledge of normal colonic motor function and mechanisms modulating it are thus limited, as is our understanding regarding the pathophysiology of colorectal disorders such as chronic constipation.

However, as described above, recent technological development has allowed great advances in our understanding of phasic motor activity acquired from the whole colon (Bampton et al., 2001, Dinning et al., 2005). Pancolonic manometry can now be used use in both paediatric and adult patients suffering with chronic intractable constipation (Dinning et al., 2010). Nevertheless, it is still considered a research tool in adults rather than a diagnostic method.

Table 1.03. Variation of colonic manometric studies performed in healthy volunteers and patients with slow transit constipation. WP: water-perfused catheter, SS: solid-state catheter, PR: per-rectal intubation with the aid of a sigmoidoscope or colonoscopy, PN: per-nasal intubation, RS: rectosigmoid, AR: anorectal, R: rectum, AC: ascending colon, TV: transverse colon, MT: mid transverse colon, DC: descending colon, AUC: area under curve, HAPC: high-amplitude propagating contraction, MI: motility index.

References	Sample		Catheter type	Intubation method	Manometric technique	Duration of study	Number of sensors	Spacing (cm)	Colonic regions studied	Study Parameters
	HV	STC								
(Preston and Lennard-Jones, 1986)	5	19	Pressure recording miniature balloons	PR	Static	1 h 30 min	3	5	RS	MI, response to Bisacodyl
(Shouler and Keighley, 1986)	22	25	WP	PR	Static	1 h	2	5	RS	MI, response to Bisacodyl
(Reynolds et al., 1987)	0	25	WP	PR	Static	2 h 30 min	2	5	RS	Response to meal
(Waldron et al., 1988)	16	44	SS	PR	Static	2 h	4	5	RS	Response to morphine, MI
(Kamm et al., 1992b)	12	8	SS	PR	Ambulant	24 h	2	10	AR	RMC
(Ferrara et al., 1994)	12	11	WP	PR	Ambulant	24 h	?	?	AR	MI, response to meal
(Bassotti et al., 1992a)	29	15	WP	PR	Static	5 h	8	12	HF R	MI, PS, HAPS, response to meal

(Bassotti et al., 1993a)	18	16	WP	PR	Static	24 h	9	12	SF R	HAPC
(Bassotti et al., 1994a)	18	16	WP	PR	Static	24 h	8	12	DT- R	HAPC
(O'Brien et al., 1996)	15	15	WP	PR	Static	3 h	5	5	SF – R	MI, HAPS
(Bassotti et al., 1999c)	0	25	WP	PR	Static	4 h	8	12	T - R	HAPC, response to Bisacodyl
(Leroi et al., 2000)	7	7	WP	PR	Static	23 h	8	10	AC - C	HAPC, response to Bisacodyl
(Herve et al., 2001)	6	6	WP	RP	Static	22 h	8	10	DC - R	HAPC, AUC, response to meal, response to Bisacodyl
(Rao et al., 2001a)	11	9	SS	PN & PR	Ambulant	30 h	6	Variable	Distal TC - R	RMC, HAPC, MI
(Hagger et al., 2003)	10	8	SS	PR	Ambulant	24 h	10	15	C - R	HAPS, MI
(De Schryver et al., 2003)	10	10	WP	PR	Static	6 h	12	1 - 10	MT - R	HAPC, AUC
(Bassotti et al., 2003c)	16	29	WP	PR	Static	24 h	8	12	HF - R	PS, HAPS
(Bassotti et al., 2003b)	14	35	WP	PR	Static	24 h	8	12	MT - R	PS, HAPS
(Bassotti et al., 2003a)	0	26	WP	PR	Static	24 h	8	12	MT - R	Cyclical activity
(Herve et al., 2004)	20	40	WP	PR	Static	24 h	12	10	MT - R	HAPS, AUC
(Rao et al., 2004)	20	21	SS	PR	Ambulant	24 h	6	7 - 15	MT - R	HAPS, AUC

1.6.1.3.1.3. Available equipment

The types of pressure-sensing catheters used for recording intracolonic contractile activity are of great significance. To date, two types of recording catheters have been employed, each with advantages and disadvantages.

A. Water-perfused catheters

These are multi-lumen catheters, made from silicone rubber, polyvinylchloride or similar materials. Such materials provide flexibility, which enhances subject tolerability during colonic intubation (either per-nasal or per-rectal, see below) (Scott, 2003, Dinning et al., 2010). However, design is not standardised and varies according to the positions (spatial resolution), number and orientation of sideholes. The maximum number of sideholes within such catheters is currently limited to 16 in studies performed by the Australian group (Bampton et al., 2001, Bampton et al., 2002, Dinning et al., 1999, Dinning et al., 2004); such studies provided multichannel recording covering the whole colonic length with side hole spacings of 7.5 cm (Scott, 2003, Dinning et al., 2009a, Dinning et al., 2010). As the distance between two adjacent recording pressure sensors decreases, more precise information about intracolonic activity can be achieved. For recording purposes, each sidehole is linked to an external pressure transducer. Distilled water from a high-pressure external reservoir is continuously perfused at a constant rate through each channel, which also varies between studies and lacks standardization (the range of perfusate rate is 0.1 - 0.6 ml/min from each side hole) (Scott, 2003). Contraction of the colonic wall will occlude the sideholes and thus generate a resistance to the flow of perfusate. This change in the resistance will be translated as pressure changes recorded by the pressure transducer (Scott, 2003).

The main limitation of using such catheters is the need for continuous water perfusion. This may load the colon with up to 4 litres of water during a prolonged study (Debongnie and Phillips, 1978), which cannot be regarded as a normal physiological state. Although the colon has a good ability to absorb water, the effect of such volumes of perfusate on contractile activity is not clear and not fully investigated. Furthermore, the subject is connected to the perfusion system via the catheter during the study period, which limits subject movement and daily activities that might also influence colonic motor activity. Nevertheless, these catheters are

highly versatile, cost-effective, and reusable after effective sterilisation (Scott, 2003, Dinning et al., 2010). Therefore, most of the existing colonic motility studies have used this type of catheter.

B. Solid-state catheters

Such catheters can host multiple miniature strain-gauge pressure sensors that are incorporated within the design of a flexible, so-called 'solid-state' catheter, and therefore does not require continuous fluid perfusion in order to record intraluminal pressure changes (Figure 1.10).

For recording purposes, each strain gauge is linked to a miniature flexible pressure-sensitive diaphragm, which forms one arm of an electronic circuit that is linked to the amplification/recording system (Scott, 2003). Deformation of the diaphragm due to changes in intra-luminal pressure cause changes in resistance within the stain gauge circuit. The larger the contraction (or relaxation) the greater the change in resistance. Accordingly, this method provides a precise tool for measurement of such activity. At present, solid-state catheters can host 3 - 10 pressure sensors with spacings of 5 to 45 cm (Scott, 2003, Dinning et al., 2010). Recording from solid-state catheters can be transmitted to portable digital recorders with large memory capacity that can then be subsequently downloaded to a computer for data display and analysis. This provides an ambulatory method for the subject under investigation for studying colonic activity over long periods, making it more acceptable; also, the data obtained will have been acquired in a more physiological manner (i.e. no water perfusion). However, such catheters are expensive; pressure transducers are less robust; and there is limitation in terms of the number of recording sensors that may be used (the more sensors, the higher the risk of catheter breakage during placement and recording). Furthermore, long solid-state catheters required for prolonged pancolonic motility studies can only be introduced by the aid of a colonoscopic technique.

To date, only the study by Soffer *et al* has been performed to record pan-colonic contractile activity and using this type of catheter; however, the study employed only 3 pressure-recording sensors (Soffer et al., 1989). Whether colonic contractile activities recorded by different types of catheters (solid-state versus water-perfused) are comparable, remains unknown.

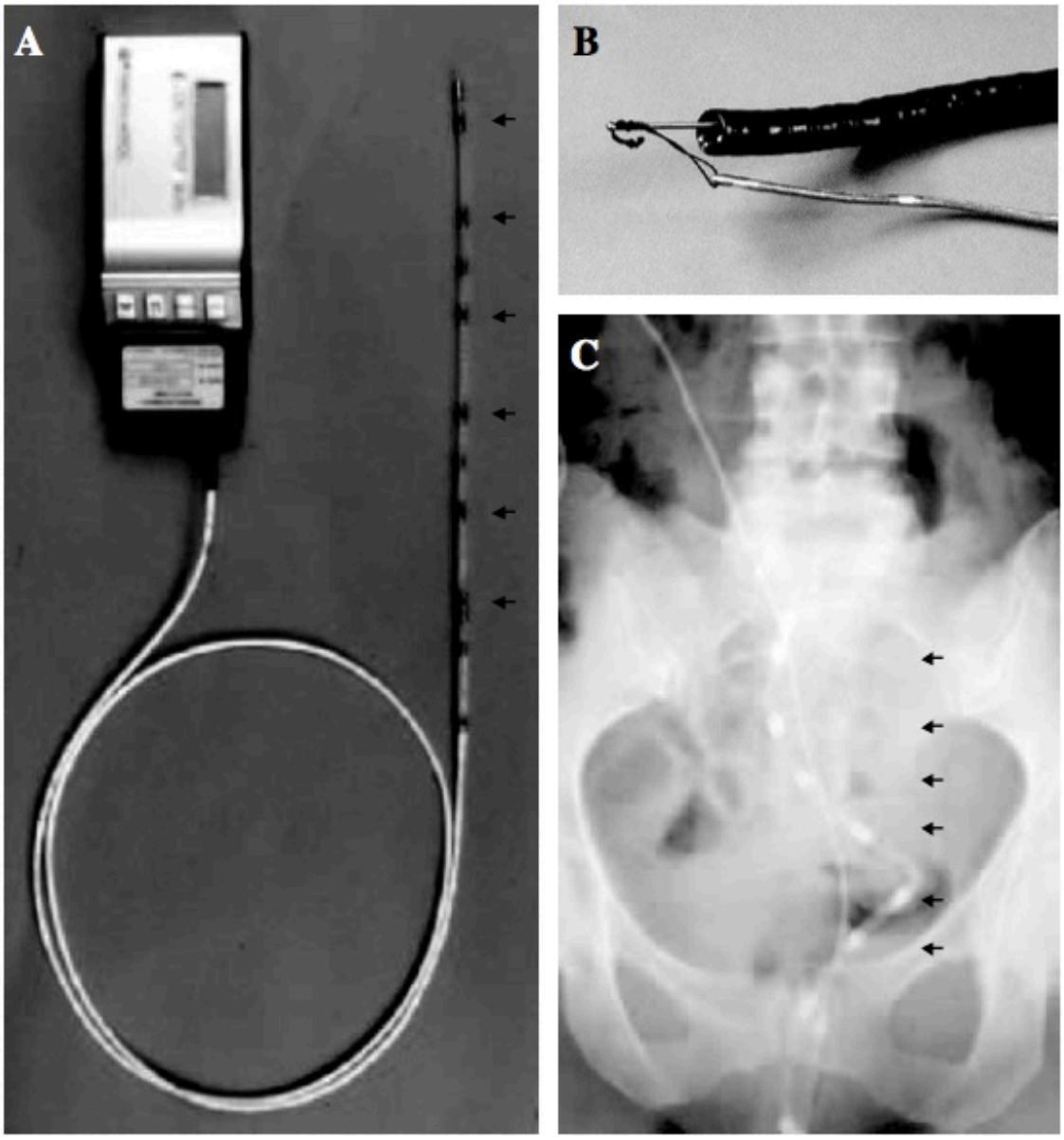


Figure 1.10. Solid-state manometric recording equipment. (**A**) six-channel solid-state catheter attached to a portable recorder with 6 microtransducer pressure sensors are arrowed; (**B**) a strong silk thread attached to the tip of the catheter and grasped by biopsy forceps to facilitate colonoscopic-assisted intubation; (**C**) plain radiograph of the catheter *in situ* in the left colon with radio-opaque pressure sensors (arrowed). (Images adapted from Scott (Scott, 2003) with permission).

1.6.1.3.1.4. Procedures of colonic intubation

Two routes of intubation may be used to place colonic manometry catheters in human studies: antegrade (per-nasal), retrograde (per-rectal), or even both routes (Scott, 2003, Dinning et al., 2009a, Dinning et al., 2010). Furthermore, intubation can be achieved through stomas (e.g. appendicectomy, caecostomy, ileostomy or colostomy) (Garcia et al., 1991). Each principal method has its own advantages and disadvantages. Retrograde intubation is achieved with colonoscopic or sigmodioscopic assistance, based on the length of colon to be studied. In contrast to antegrade intubation, retrograde intubation usually requires bowel preparation, particularly for longer study segments. Per-nasal intubation is usually performed under fluoroscopic guidance up to the pylorus. The catheter is then gradually fed to the study point of interest. Therefore, per-nasal intubation is time consuming, especially in constipated patients in whom progression of the tip of the catheter to the desired site may take several days (Dinning et al., 2010). In addition, per-nasal intubation can only be performed with the use of the flexible water-perfused catheters. However, the main advantage of the per-nasal route is the lack of need for prior bowel preparation, which allows for a more physiological recording technique.

1.6.1.3.1.5. Protocols of Colonic manometry

To date, there has been a lack of standardisation as to the study protocol employed in most colonic manometric studies performed in both healthy controls and patients.

1. Duration of recording: although a 24 h recording period may be required to appreciate circadian rhythms of the colon. Most studies carried out thus have been short duration (up to 8 h) (Scott, 2003); a few prolonged recordings have been performed using water-perfused catheters (Narducci et al., 1987, Bassotti et al., 1988, Jouet et al., 1998, Bampton et al., 2000, Bampton et al., 2001). A recovery period (usually 2 - 24 h) following colonic intubation may be required if prior bowel preparation and sedation have been used, which may affect colonic contractility. The duration of the recovery period is dependent on the extent of colonic intubation (more proximal intubations require longer recovery periods), and also the half-life of sedative medications or other medication used to aid colonic intubation.

2. Recording of physiological colonic activities: ideally, prolonged manometric studies should record colonic contractions during daytime and at night, and include 2 - 3 meals. Ideally, colonic activity may be recorded during defaecation, if this occurs during the study period.

3. Caloric stimulation: meal composition should be standardised, usually with high calorie meals (average >400 - 1000 kcal) required to induce a sufficient colonic response (gastro-colonic response) (Scott, 2003). Water should be allowed during the study period as required; however, coffee (Rao et al., 1998b), smoking (Jameson and Misiewicz, 1993, Meier et al., 1995) and alcohol consumption (Berenson and Avner, 1981) should not be allowed due to their reported effects on colonic motility.

4. Provocation test: this type of test has usually been performed in short duration studies when recording of colonic contractile activities before (basal state), during, and after a stimulus is performed. The most common examples are:

(a) pharmacological and chemical stimulation: the effect of various agents and chemical compounds on colonic contractility can be studied using a colonic manometric technique. Most studies thus for have aimed to investigate colonic neuronal control in addition to the direct effect of such agents on colonic movements. The most common route of administration is direct intraluminal infusion into the colonic lumen via an endoscopically placed tube or by direct rectal administration, although oral and intravenous routes have also been used. The effects of various laxatives and other agents on inducing colonic motor activity have been studied in constipated patients with a few studies involving healthy controls e.g. rectal or colonic infusion of bisacodyl, chenodeoxycholic acid and intravenous injections of edrophonium chloride (an anticholinesterase agent) (Dinning et al., 2005, Bassotti et al., 1993a, De Schryver et al., 2003, Leroi et al., 2000, Kamm et al., 1992b, Bassotti et al., 1999a).

(b) distension stimulation: The effect of colonic intra-balloon distention on initiating colonic contractile activities has been poorly studied, with conflicting results.

(c) external stress stimuli: the effect of various stress stimuli (fear of inescapable foot shock, water avoidance, tail shock, loud noises, cold environment) have been studied widely in animal studies. These show that such stimuli increase colonic

motility and frequency of defaecation (Enck and Holtmann, 1992). However, only a few studies have examined the effect of psychological and physical stress on colonic motility in healthy humans, and all have been limited to distal colonic regions. Examples of these stimuli include stressful interview, dichotomous listening test, fright or anger, intelligence test, and the cold-water immersion test (Almy, 1951, Narducci et al., 1985, Welgan et al., 1988).

1.6.1.3.1.6. Data analysis

Interpretation of manometric recordings is complex due to wide quantitative and qualitative variability of recorded pressure changes, which are usually yielded from multiple recording sites and over prolonged periods. Various types of artifacts are recorded during the study, which may be the result of straining, respiration, change in body position, coughing, fault in pressure sensors etc. In comparison to manometric recordings obtained from the upper GI tract, colonic motility tracings usually show more artifacts, shifts in baseline, and more complex pressure wave forms (De Schryver et al., 2002). There remains lack of standardisation of criteria used to define individual or grouped pressure wave characteristics. A list of the parameters used in the analysis of colonic manometric recordings is summarised below (Table 1.04).

Colonic contraction parameters

- Qualitative analysis
  - Type of contractile activities
    - high and low amplitude contractions
    - propagative and non-propagative contractions
    - 'isolated or in 'patterns'
- Quantitative analysis
  - Individual contractile activities
    - number of contractions
    - frequency of contractions
    - duration of contractions
    - amplitude of contractions
    - colonic site of origin
    - velocity of contractions
    - direction (orad, aborad)
    - length of propagation
  - Colonic contractility patterns
    - number of contractions per unit time
    - duration of time occupied by contractile activity
    - motility index (MI)
    - area under the pressure curve (AUC)

Table 1.04. Colonic contraction parameters measured during qualitative and quantitative data analysis. Further criteria for the identification of various contractile patterns are described later (Table 1.05 and Figure 1.11).

Currently, two main methods are used to evaluate data obtained from colonic manometric recordings: manual and computer-based analyses. For each method, baseline detection, artifact elimination and peak detection should be performed (Scott, 2003). Pressure waves are identified from each recording channel relative to the channel-specific baseline and compare to other pressure waves recorded within same or adjacent channels (to appreciate regional variation) (Scott, 2003).

A. Manual (visual) analysis

Most early studies were observational in nature and therefore they only used broad descriptive terms to describe colonic contractile activities (Templeton and Lawson, 1931, Adler, 1941, Quigley, 1950, Spriggs et al., 1951, Connell, 1961, Ritchie et al., 1962, Ritchie et al., 1968). More recent studies have described patterns of colonic activity and classified colonic pressure waves on the basis of their characteristics (Narducci et al., 1987, Bassotti et al., 1988, Soffer et al., 1989, Moreno-Osset et al., 1989, Cook et al., 2000, Bampton et al., 2001). However, technique of visual analysis is extremely time-consuming, and subject to observer bias and inter-observer variation. Nevertheless, visual analysis is important when analysing recorded symptoms and linking them to specific pressure events registered during the study period.

B. Automated (computer software-based) analysis

This allows for standardisation, simplification, and removal of many visual analysis confounders from manometric recordings. However, few specific computer-based algorithms are available to analyse colonic motor activities (Parker et al., 1987, Rogers and Misiewicz, 1989). The most recent study by De Schryver *et al* concluded that 97% of all pressure peaks detected by the human eye were also recognised by computer software. Conversely, 92% of automatically-detected peaks were also observed by human investigators (De Schryver et al., 2002). However, the validity of such computer-based analysis has not been formerly tested nor compared to human-based analysis in clinical studies.

1.6.1.3.1.7. Phasic colonic contractile activities as defined by colonic manometric studies

Different types of phasic colonic contractions can be detected at the same time and/or within the same unit of area; these are defined by differing peak amplitudes and durations of contractions. The main aspects of colonic contractile activity that can be evaluated using manometric techniques are summarised in table 1.05.

Unfortunately, there are no universally accepted definitions or recognised nomenclature for phasic colonic contractile activities. Based on early observations and also 24 h manometric recordings in healthy subjects, colonic motility in the basal state is principally represented by segmental (mixing) contractions and also propulsive contractile activities (Adler, 1941, Spriggs et al., 1951, Ritchie et al., 1962, Torsoli et al., 1971, Cook et al., 2000, Bampton et al., 2001, Rao et al., 2001b, Bassotti et al., 1993b, Bassotti et al., 1999c). These patterns have been sub-classified and based on radiological assessment of colonic motility performed decades ago (Ritchie et al., 1968, Ritchie et al., 1971). A summary of this classification is shown in Table (1.05) and Figure (1.11).

Table 1.05. Types of colonic phasic contractile activities as described in literature. LAPC= low-amplitude propagated contractions; PS= propagating sequence; HAPC= high-amplitude propagated contractions; HAPS= high-amplitude propagating sequence; CMC= colonic motor complexes; PCMA= periodic colonic motor activity; PRMA= periodic rectal motor activity. [Adopted from (Scott, 2003)].

Type of phasic activity	**Propagation**	**Occurrence**	**Contraction type**	**Amplitude (mmHg)**	**Duration (seconds)**	**Velocity (cm per sec)**	**Frequency (contractions per minute^{-1})**	**Occurrence (*per* h)**
segmental	Non propagating or propagating for short period	Single	Short duration/ Long duration	5 - 60	15 - 60	-	-	20 - 50
		Bursts	Rhythmic/ Arrhythmic	-	<15	-	-	
propulsive	Propagating over long distance	Single or within propagated sequence	LAPC, PS, HAPC, HAPS	20 - 80 100 - 180	≈10 10 - 20	1 – 2 0.7 – 1.2	- -	1.9 - 5 0.2 - 0.6
organised 'patterns'	Non propagating or propagating over short period	Periodic bursts	CMCs/PCMA RMCs/PRMA	- 5 - 60	6 - 8 (min) 3 - 30 (min)	- -	3 – 6 2 - 8	0.6 - 2 0.2 - 1.4

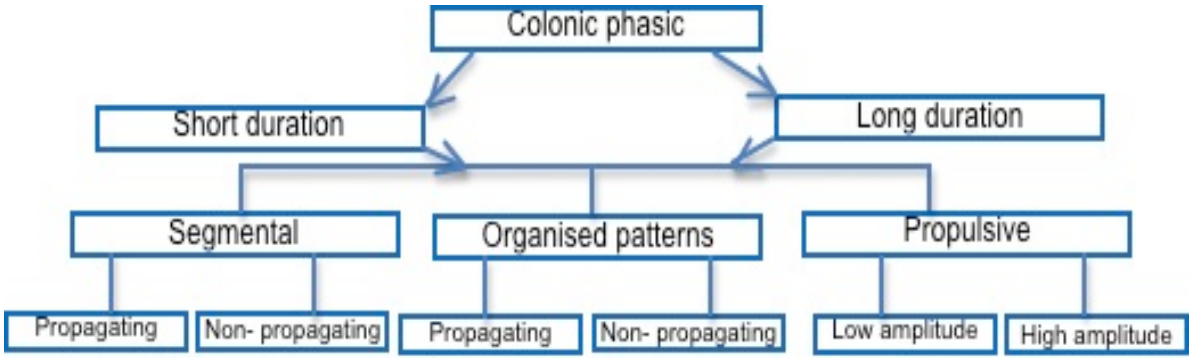


Figure 1.11. Schematic overview of the current classification of colonic phasic contractile activities identified using colonic manometric techniques based on prolonged manometric and radiological colonic motility studies.

A. Segmental contractions

Manometric studies combined with radiological investigations or transit studies have shown that colonic motility in the basal (fasted) state is mostly dominated by segmenting contractions that comprise the highest proportion of recorded colonic motor activity (Ritchie et al., 1962, Ritchie et al., 1968, Ritchie, 1971, Hardcastle and Mann, 1968, Torsoli et al., 1971, Bassotti et al., 2005). These contractions appear to be localised to one or two adjacent recording sites (i.e. propagation of <15 cm), and are not associated with mass movement of intraluminal content as determined by prolonged manometric studies (Scott, 2003, Soffer et al., 1989, Narducci et al., 1987). Propagating segmental contractions are usually of short duration and of amplitude ranging from 5 mmHg to 50 mmHg. These occur mainly as isolated contractions or, less frequently, as arrhythmic bursts of contractions (Bassotti et al., 2005). Their function appears to be to slow colonic transit and allow mixing of intraluminal contents, and provide maximal contact with the luminal lining to achieve sufficient water and electrolyte absorption (Bassotti et al., 2005).

B. Propulsive contractions

Propulsive colonic activities were described in early radiological studies as infrequent vigorous contractions that are able to push colonic content over long distance (termed “mass movement”) (Holzknecht, 1909b, Barclay, 1912a). Such observations were confirmed later with combined manometric and radiological studies (Ritchie et

al., 1962, Hardcastle and Mann, 1968, Torsoli et al., 1971, Bazzocchi et al., 1991, Cook et al., 2000). Colonic propagated events are currently defined as pressure waves migrating over >3 adjacent recording pressure channels (or more than 15 cm) at a velocity of 0.2 - 12 cm/sec (Scott, 2003). However, such a definition is highly dependent on the spacing between pressure sensors within the manometric catheter, which can hugely vary (as shown before in Table 1.02). Propulsive waves can be most simply subdivided into: high amplitude (HAPC) (Figure 1.12) and low amplitude (LAPC) propagating pressure contractions. These pressure waves have been called propagating sequences (PS) and can either move in an antegrade or retrograde direction.

HAPC are equivalent to 'mass movement' pressure waves described earlier. HAPC have received the greater attention, despite them being an infrequent phenomena, due to the simplicity of recognising these contractions. They occur, on average, 3 - 6 times per day, with an average amplitude of 50 - 116 mmHg, which is almost homogenous along the entire colon (Scott, 2003). Details of existing definitions for HAPC have previously been reviewed (Scott, 2003). HAPC are clearly associated with physiological events such as faecal expulsion (Bampton et al., 2000, Bharucha, 2012), but they can also be induced by pharmacological stimulation and colonic distension (Bharucha, 2012). HAPC are also more frequent after morning awakening, during daytime, following meals and after exercise (Crowell et al., 1991, Bassotti et al., 1994b, Cook et al., 2000).

LAPC are poorly described in man despite representing the predominant colonic contractions recorded by manometry. They are propagated waves of amplitude 5 - 40 mmHg, with a frequency of 45 - 120 times per 24 h (Scott, 2003). There is clear overlap with the definition of both HAPC and LAPC influenced by methodology used. Previous studies suggest that LAPC are involved in the transport of mainly liquid colonic contents (Chauve et al., 1976, Gattuso et al., 1996), and are also associated with the passage of flatus (Bassotti et al., 1996). Furthermore, a recent combined manometric and scintigraphic study showed that in healthy volunteers, low and high amplitude contractions were equally effective at transporting isotope in the proximal colon (Dinning et al., 2008a).

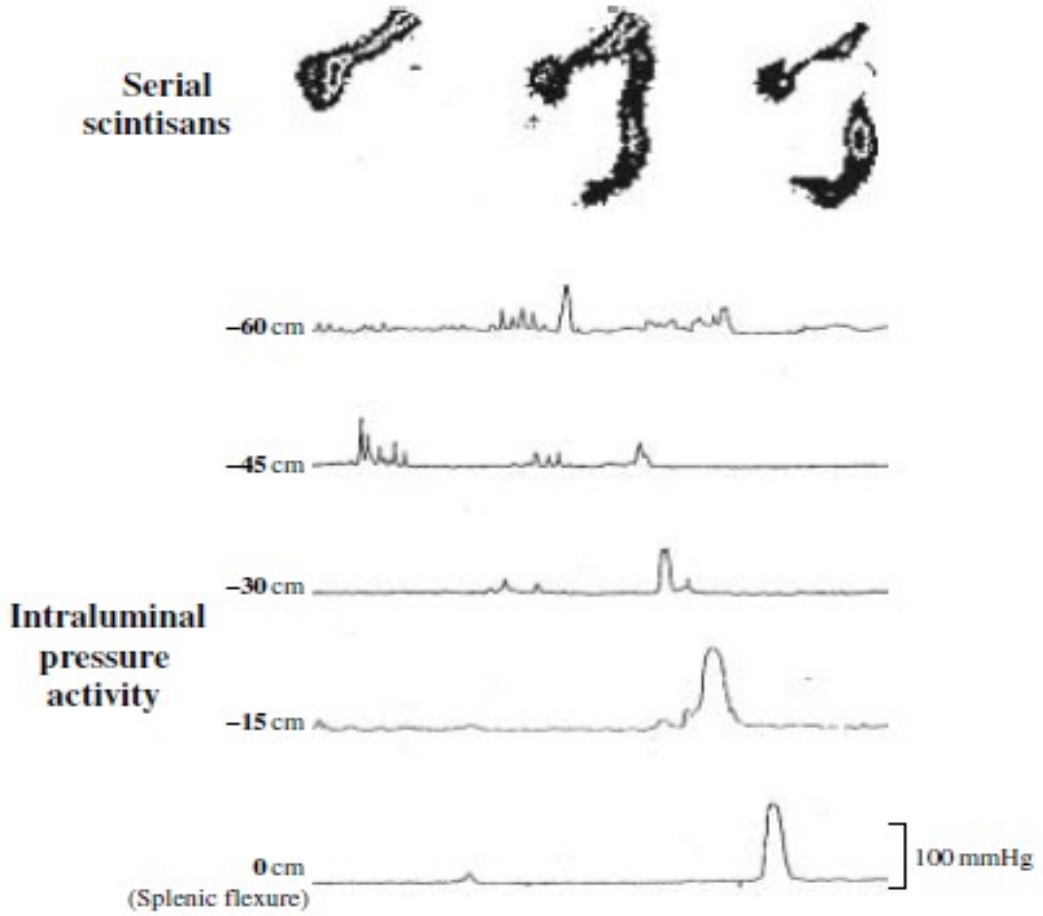


Figure (1.12): Colonic 'mass movement': simultaneous assessment of intraluminal pressure change recorded by manometry and transit as defined by a scintigraphic technique. The lower five tracings represent a 3 min period of intracolonic pressure activity (as recorded via a perfused manometry catheter), over a 60 cm study segment proximal to the splenic flexure. A high amplitude (>100 mmHg) propagating contraction (HAPC), originating in the ascending colon, migrates rapidly (1 cm s^{-1}) to the splenic flexure; this is concomitant with a marked shift in intraluminal contents, as seen by movement of the radio labelled marker (^{99}Tc, previously instilled in the caecum) from the transverse to the descending colon. Three serial (1 min) scintiscans are shown in the upper part of the figure [Adopted from (Scott, 2003)].

C. Organised groups of contractions (motility 'patterns')

It was previously assumed that the human colon lacks the characteristic cyclical motor activity, as described in the small intestine, where it is known as the migrating motor complex (MMC) (Wingate, 1981, Sarna, 1985), or within the rectum (rectal

motor complex: RMC) (Dinoso et al., 1983, Kumar et al., 1989, Orkin et al., 1989). The small intestinal and rectal motor complexes have been compared in ambulatory healthy subjects, in term of their contraction characteristics and whether they are linked (Kumar et al., 1990, Prior et al., 1991, Kurakake et al., 1993).

RMCs are defined as cyclical bursts of phasic contractions; these exhibit specific characteristics as shown below in table 1.06.

Rectal Motor Complexes (RMCs)
• They are different from the MMC of the upper gut
• They have a cyclical rhythm, and are predominant in the nocturnal period
• The duration of each RMC is >3 minutes
• Contraction frequency is >2 per minute
• Amplitude is 15 - 60 mmHg
• They appear to be non-propagating in nature

Table 1.06. Characteristics of rectal motor complexes as identified in studies performed in healthy humans (Dinoso et al., 1983, Kumar et al., 1989, Orkin et al., 1989, Prior et al., 1991, Kurakake et al., 1993, Herbst et al., 1997, Rao and Welcher, 1996, Rao et al., 1998a, Rao et al., 2001b, Spencer, 2001, Chan et al., 2005).

It is now clear that more proximal regions of the human colon also display regular cyclical motility patterns, called colonic motor complexes (CMCs). This is akin to most mammalian species (Scott, 2003). CMCs are defined as bursts of phasic pressure activity, of >8 mmHg, and >3 min duration, that recur at periodic intervals (Scott, 2003). Such activities appear to have been missed during analysis of colonic contractile recordings until recently Bampton *et al* and Hagger *et al* performed prolonged recordings of colonic motility using long water-perfused catheters introduced via a per-nasal route without any prior bowel preparation; they reported the presence of regular CMCs which occur once to twice per hour during the day and at night time (Bampton et al., 2001, Hagger et al., 2002). Given that most of the

earlier prolonged studies of pancolonic activities had involved prior bowel preparation, it is possible that this may have had some affect on the contractile 'patterns' seen. However, the effect of bowel preparation on colonic motility in healthy human has not been formally investigated.

The function of periodic colorectal motor activity appears to be to facilitate mixing of intraluminal content and probably aid in propulsion (over short distances); however, they do not exhibit the lumen clearing function of the small bowel MMC (Scott, 2003). Such periodic activities are also believed to be controlled by the enteric nervous system and reflect its integrity (Spencer, 2001); however, the detailed underlying regulatory mechanisms are poorly understood. The association between colorectal periodic activities (i.e. RMCs and CMCs) is also unknown.

The process of defaecation also involves stereotypical and organised motor activity, in that it is accompanied by an increase in antegrade propagating sequences (PS) especially within the predefaecatory phase, an hour prior to faecal expulsion (Bampton et al., 2000). These sequences usually originate in the proximal colon and progress toward the distal colon. The situation is reversed prior to defaecation, when PS initially originate more distally, with subsequent PS arising more proximally (Bampton et al., 2000).

1.6.1.3.1.8. Colonic manometric studies in STC

In health, prolonged manometry studies have revealed that colonic motor activity is 'complex, intermittent, and variable across colonic segments, and exhibits temporal and spatial variation' (Singh et al., 2013). Limited data are available in the literature with regard to pancolonic motor function in patients with STC. In fact, of the approximately 20 manometric studies reported in STC (Table 1.03), the majority were limited to the rectosigmoid and left colonic regions. Very few have compared STC to other constipation subgroups (Bassotti et al., 1993a, O'Brien et al., 1996, Bassotti et al., 2003b). Also, criteria for inclusion was notably inconsistent; for example, Herve *et al* defined STC as a transit time greater than 70 h (Herve et al., 2004), whilst Rao *et al* defined STC as >20 % of markers retained at 120 h (Rao et al., 2001a). There is also great variability among these studies in term of methodology, technique, catheters type, placement of catheters, and parameters adopted for data analysis (Table 1.03). Of those studies performed in the

rectosigmoid region, most did not use prior bowel preparation and few were prolonged (for 24 h). Conversely, of those studies designed to assess more proximal colonic segments, partial or complete bowel cleansing was employed to facilitate colonoscopic placement of the catheter. Furthermore, most studies adopted water-perfused manometric techniques; very few used solid-state catheters and were ambulatory in nature (Kamm et al., 1992a, Ferrara et al., 1994, Rao et al., 2001a, Rao et al., 2004, Hagger et al., 2003). All studies employed either a per-rectal (with the aid of sigmoidoscope or colonoscopy) or per-nasal route of intubation, except one by Hagger *et al* who used both techniques to cover the whole colon (Hagger et al., 2003). Finally, given that STC almost exclusively occurs in women; over 90% of patients enrolled in those studies were female.

Nevertheless, regardless of the methods used, these studies provide insight into colonic motor activities in STC, and their findings are summarised below.

Rectosigmoid manometric studies: these include all studies where the catheter tip had not progressed beyond the sigmoid colon. Findings are inconsistent: some report a reduction in overall motility (Waldron et al., 1990, Ferrara et al., 1994), response to a meal (Reynolds et al., 1987, Ferrara et al., 1994), response to awakening (Ferrara et al., 1994), and response to intrarectal administration of bisacodyl (Preston and Lennard-Jones, 1985, Shouler and Keighley, 1986). By contrast, other studies have shown normal basal levels of rectosigmoid motility in STC (Shouler and Keighley, 1986, Reynolds et al., 1987, Waldron et al., 1988), or normal response to bisacodyl (Kamm et al., 1992a).

Colonic manometric studies: none of the studies performed in chronic constipation or STC have been pancolonic in nature (from caecum to anal canal). In fact, in all previous prolonged manometric studies, the tip of the catheter was rarely progressed beyond the proximal portion of transverse colon. These studies in general concentrate on frequency of recorded HAPC (neglecting other recognised colonic motor activities) and have shown an overall reduction in the number, amplitude and duration of HAPC in patients with STC (Bassotti et al., 1992b, Bassotti et al., 1993a, Bassotti et al., 1994b, Bassotti et al., 1999b). Similar findings have been shown in patients with chronic idiopathic constipation (not specifically STC) (Kamm et al., 1988, Leroi et al., 2000), obstructed defaecation (Dinning et al., 2004), and also in

normal transit constipation (Bassotti et al., 1994a), suggesting that abnormalities in HAPC are not exclusive to STC. Response to meal ingestion (gastro-colonic response) has also been shown to be impaired in STC (Kamm et al., 1988, Bassotti et al., 1992b, De Schryver et al., 2003) and in other patients suffering from chronic idiopathic constipation (Leroi et al., 2000). Contrasting results have been published with regard to the motor response to sudden awakening. Some show no change (Bassotti et al., 1999c), while others report a diminished response (Rao et al., 2004). Circadian rhythm is reported to be similar in STC and healthy volunteers (Rao et al., 2004). Colonic motor response to intraluminal bisacodyl has been reported to be reduced in some patients with STC (Leroi et al., 2000, De Schryver et al., 2003). This observation, has, however, been refuted by other studies of patients with severe idiopathic constipation (Kamm et al., 1992a) and with proven STC, in which, the majority (88%) had an intact bisacodyl response. Motor responses to intravenous administration of the anticholinesterase agent, edrophonium, are also reduced in patients with STC (Bassotti et al., 1993a).

1.6.1.3.2. Tonic colonic motor activities

Tonic contractions are defined as sustained and more prolonged contractions of gut segments compared to phasic contractions. Tonic colonic contractions (which maintain wall ‘tone’) also result from smooth muscle activity. Tonic changes cannot be assessed with the use of thin colonic manometric catheters, as direct contact with the bowel wall is required. Tonic activity is a reflection of colonic wall biomechanical (colorectal capacity) and elastic (distensibility) properties and contributes to wall ‘compliance’.

Colorectal compliance reflects the ability of the colon to expand in response to an imposed force, which leads to changes in intraluminal pressures (Whitehead and Delvaux, 1997). For large changes in intraluminal pressure with small volumes, the stiffer the colorectum is (i.e. hypocompliance). Conversely, small increase in pressure in response to large inflation volumes means a more lax or flexible colorectum (i.e. hypercompliance).

1.6.1.3.2. 1. Historical data

Description of human tonic colonic activities dates back to the twentieth century (Spriggs et al., 1951, Ritchie et al., 1962), where slow changes in colonic pressure were recorded. However, the first attempt to truly record colonic tone was undertaken by White *et al* using 'colonmetrogram' in patients suffering from brain and spinal injuries (White et al., 1940). This technique predated that of the 'barostat', which involves the controlled pumping of air into an infinitely compliant intraluminal bag, and monitoring intraluminal pressure and volume changes. Changes in air volume within the bag reflect volume changes within the intestinal lumen, which correspond to changes in intestinal tone (Azpiroz and Malagelada, 1985, Bell et al., 1991). Azpiroz and Malagelada first highlighted the importance of this technique in assessing GI motility in the mid 1980s (Azpiroz and Malagelada, 1985, Azpiroz and Malagelada, 1987). The barostat was validated for use in assessing colorectal motility in the early 1990s (Steadman et al., 1991, Steadman et al., 1992). Further studies using the barostat have confirmed that human colonic tone exhibits rhythmic diurnal changes (less tonic in the nocturnal period) and also more tonic after meals (Steadman et al., 1991, Ford et al., 1995).

1.6.1.3.2. 2. The existing barostat technique

This technique is computer-based and allows constant airflow in and out of an air-filled bag to be maintained by means of a feedback mechanism (air injection/ aspiration system) (Scott, 2003). The intraluminal bag should be 'infinitely' compliant (typically a very thin polyethylene bag), and for colonic studies it needs to be oversized (i.e. bigger than the organ under the study) which ensures maximum contact with the colonic wall regardless of the diameter of the studied segment (Whitehead and Delvaux, 1997, Scott, 2003). The bag is mounted to a manometric catheter with two ports for inflation and deflation, and also a pressure monitoring port (Steadman et al., 1991, Whitehead and Delvaux, 1997). However, the design of both the catheter and the attached bag varies according to study requirements and therefore is not standardised. The barostat can measure tonic and phasic contractions, biomechanical gut properties, compliance, and also visceral sensation.

1.6.1.3.2. 3. Colorectal intubation

Prior bowel preparation is usually required for performing barostat studies, especially for more proximal colonic regions. However, a rectal barostat study can be performed without prior cleansing if the rectum is confirmed on digital examination to be empty. A gentle, nonchemical enema (tap water) should be enough to ensure complete rectal emptying and avoid mucosal sensitisation. Similar intubation can be used to place a barostat bag mounted to a manometric catheter (antegrade or retrograde intubation) (Whitehead and Delvaux, 1997, Dinning et al., 1999, Coffin et al., 1999); however, retrograde intubation is easier to achieve and well tolerated by subjects. During the study, subjects should stay in the lab (non-ambulatory) and therefore, prolonged studies >8 h are rarely performed (Scott, 2003). Subjects usually lie in a semi-prone position (20 degree Trendelenburg) to reduce pelvic hydrostatic pressure (Whitehead and Delvaux, 1997).

1.6.1.3.2. 4. Protocols

Measurement of colonic phasic and tonic activity is usually performed shortly after intubation if no medications have been used; however, for a colonoscopically placed catheter, sufficient recovery period is required. Whitehead *et al* fully reviewed the method of performing barostat studies in an attempt to standardise the technique (Whitehead and Delvaux, 1997). This can be summarised as:

1. unfolding: once the bag is within the desired colonic segment, inflation of the bag to almost the maximum capacity (this can be done manually and rapidly) should be performed in order to unfold the bag, followed by deflation.

2. conditioning distention (CD): a 30 - 60 min equilibration period has been recommended to allow basal tone to stabilize, as well as to familiarise the subject to the barostat assembly (Hammer et al., 1998). However, another recent study showed that a rectal barostat study can be performed without the need to perform prior CD, without any effect on recording (Bajwa et al., 2013).

3. minimal distending pressure: following CD, the minimal pressure required to prevent the barostat bag from collapsing due to intra-abdominal pressure needs then to be measured. This can be determined by inflating the bag in small increments until respiratory excursions are recorded.

4. barostat operating pressure: this is usually set at 2 mmHg above operating pressure. The average reported operating pressure is 8 - 17 mmHg (Scott, 2003).

5. basal tone: recording of volume changes within the barostat bag should be recorded during a fasting period for a minimum of 30 - 60 min.

6. stimulation test: the effect of physiological stimuli (e.g. response to meal) or pharmacological stimuli can be performed dependent upon the study aims. Given that a barostat study is non-ambulatory and requires the subject to be connected to the recording machine at all times, the effect of awakening and sleep is rarely assessed during this technique. Defaecation is also not possible to record.

1.6.1.3.2. 5. Data analysis

Automated analysis for tonic contractions is performed through commercially available software (Scott, 2003). Data obtained from the manometric catheter (phasic activities) can also be analysed. Artifact resulting from body movement, respiration, and from the machine itself is required to be eliminated prior to analysis. This can be performed automatically by excluding all simultaneous pressure waves of <5 second duration (Bharucha et al., 1997).

1.6.1.3.2. 6. Tonic colonic contractions as defined by mechanical barostat

Recorded changes in baseline intra-bag volume reflect colorectal tone. After exclusion of phasic contractions, intra-bag volumes can be averaged over a specific time period from the start of the recording (Scott, 2003). Changes in colonic tone are usually expressed as a percentage compared to an individual's basal tone, due to large inter-subject variation (Scott, 2003). The average normal mean baseline volume for proximal colonic regions is reported to be 125 - 250 ml; for the distal colon, mean baseline volume is 60 - 160 ml (Scott, 2003). In healthy volunteers, colonic tone tends to increase following meals (Steadman et al., 1991, Jouet et al., 1998, Soffer et al., 2000) and decrease during sleep (Steadman et al., 1991).

1.6.1.3.3. Factors influencing colonic motor activities

1.6.1.3.3.1. Circadian rhythm:

Under 'normal' conditions, human colon contractile activity is subject to diurnal variation. Most prolonged manometric studies that have recorded colonic motility report that motor activity is decreased or even abolished during sleep (including an afternoon 'nap') and increased throughout the colon after awakening (Narducci et al., 1987, Kumar et al., 1989, Soffer et al., 1989, Bassotti and Morelli, 1990, Bassotti et al., 1993b, Bassotti et al., 1999c, Bampton et al., 2001, Rao et al., 2004). This may be influenced by the gut biological clock, which is controlled by neuronal and hormonal (such as melatonin) pathways (Konturek et al., 2011). The circadian characteristics of each colonic contractile activity have been described previously in their specific sections.

1.6.1.3.3.2. Aging

Colonic motility may be influenced by the ageing process; however, there is a paucity of data in elderly populations and also a lack of randomised control trials comparing colonic motor activities in various age group. This is possibly due to the relatively invasive nature of the colonic manometric technique, which makes it more challenging to perform in older subjects. A review of manometric studies performed in paediatric subjects suggest that HAPC frequency decreases with age, whereas segmental contractile activity increases (Bassotti et al., 1999c). This is supported by animal studies showing that such changes in colonic motility may be related to alterations in colonic smooth muscle or its regulatory neurons (Takahashi et al., 2000, Wade, 2002). One study has investigated the effect of ageing on rectal tone and compliance in two groups of healthy volunteers (group 1: 70 - 94 yr. vs. group 2: 23 - 28 yr.); they reported no significant difference in rectal barostat bag volumes both in fasting and postprandial periods (Lagier et al., 1999).

1.6.1.3.3.3. Gender

Variations of colonic motor activities between genders are also poorly understood. There are no studies in the literature directly assessing the differences in colonic motor function between men and women. Similarly, effects of dietary intake and physical activities on motor activities are also poorly defined with no sufficient data in the available literature.

1.6.2. THE CONTROL OF COLONIC MOTILITY

The control of colonic motility is not fully understood. There are three main regulatory mechanisms that are interlinked and co-operate to regulate colonic myoelectrical and motor activities, and hence transit.

1.6.2.1. Myogenic control

The muscular filaments (actin and myosin) lie within the circular and longitudinal muscle fibres and are responsible for producing chemical energy, resulting in muscular fibre shortening. In spite of the fact that both circular and longitudinal muscle layers have different myoelectric and motor activities as shown by *in vitro* studies, their functions are highly interlinked (Huizinga et al., 1983, El-Sharkawy, 1983, Liu and Huizinga, 1993). The presence of intestinal contents along with neuronal activity induces slow wave myoelectric activity occurring at frequencies between 2 - 13/min (Sarna, 1991). Slow waves are generated through interstitial cells of Cajal (ICCs) (Huizinga et al., 1995, Sanders, 1996). The importance of ICCs for the integrity of motor function of the gut, and their role in gut dysmotility has prompted major research in this field. The reduction or loss of ICC populations can be found in some cases of chronic constipation and other gut dysmotility disorders (Lyford et al., 2002, Huizinga et al., 2009). However, coexistent neuronal damage is also reported in such cases, which indicate that these cells probably work in conjunction with other regulatory mechanisms rather than as a separate entity.

1.6.2.2. Neuronal control

Neural control mechanisms are integral to the generation of propulsive activity in the gastrointestinal tract. Afferent nerves generate and convey, via specialised endings, visceral sensory information. Efferent neurons have a role in controlling gut motility but also have more modulatory roles in secretory and absorptive functions. The

neural control of the colon, like the rest of the gastrointestinal tract, is believed to be provided by the three neuronal components: the enteric nervous system (ENS), autonomic nervous system (ANS), and central nervous system (CNS) (Wingate, 1993).

Colonic innervation can further be sub-classified into two components: (a) intrinsic neurons (ENS), which are intramural, and lie in plexuses which consist of ganglia (clusters of tightly packed nerve cell bodies and glial cells), connected by intraganglionic fascicles of nerve fibres arising principally from the nerve cells (myenteric and submucosal plexus and submucosal neuronal layers). The ENS play a role in controlling gut motility, exocrine and endocrine secretions and microcirculation of the GI tract (Furness and Costa, 1987, Wingate, 1993); (b) extrinsic neurones whose cell bodies lie extra-intestinally, though they may subsequently follow an intramural course (Camilleri and Ford, 1998). Extrinsic neurons are generally considered to be part of the ANS, which is in turn influenced by the CNS.

The ENS (mainly within the myenteric plexus) is known to provide continuous control of all spatial and temporal colonic contractions, whereas the ANS and CNS mainly modulate ENS activity, apart from during defaecation, where the CNS input is transmitted directly through autonomic nerves (Sarna, 1991, Wingate, 1993, Bassotti et al., 1995, Camilleri and Ford, 1998). The ENS participates in peristaltic reflex activity, which is of particular importance to the generation of propulsive contractile activity. Neural control provides the necessary stimulation, by the release of neurotransmitters, for the depolarisation of muscular membranes resulting in a series of complex electrical signals, which control propulsive contractions.

1.6.2.3 Chemical control

Various chemicals and neurotransmitters are secreted from nerve endings and endocrine-paracrine cells within the gut wall, which may act directly on smooth muscle fibres, pre-synaptic, post-synaptic enteric neurones, ganglia, the spinal cord, or the central nervous system, to induce (excitatory effect) or inhibit (inhibitory effect) colonic motor activity. However, a review of all substance reported to alter colonic motility and their mechanism of action is beyond the scope of this thesis.

1.7. KNOWLEDGE GAPS OF COLONIC MOTOR FUNCTION IN HEALTH AND SLOW TRANSIT CONSTIPATION

Little is known about the pathophysiology of colonic dysmotility. In order to better appreciate colonic motor function, direct observation using colonic manometric techniques rather than indirect assessment by studies of intra-luminal transit are required. One of the most evident gaps in our knowledge of colonic motor function is the definition of normal pan-colonic activity, as most studies have concentrated on distal colonic regions rather than studying the whole colon. Prolonged studies of pan-colonic motor activities during sleep, fasting, postprandial periods, after awakening, and during defaecation are required to better understand colonic motor physiology, and subsequently pathophysiology. Furthermore, there is a lack of standardisation with regard to recording technique, and the effect of such variation is unknown. Significant advances in our understanding of the propulsive activities exhibited by the human colon in health and disease have been achieved recently, and are now possible with the aid of recording techniques using long manometric catheters that span the whole length of the colon.

1.8. CLINICAL RELEVANCE OF COLONIC DYSMOTILITY AND DISORDERED DEFAECATION

There are several examples of common and highly prevalent conditions where disturbances of colonic motor activity may interfere with colonic function, resulting in alteration of normal patterns of defaecation. Many of these conditions have been considered to be ‘functional’ or psychological disorders, due to lack of clear underlying pathophysiological abnormalities (Dinning and Di Lorenzo, 2011, Dinning et al., 2009a, Bassotti et al., 1988, Bassotti et al., 1992b, Rao et al., 2004, Patton et al., 2013, Quigley, 2010). Examples of such conditions are functional diarrhoea, functional constipation, faecal incontinence, abdominal bloating, and irritable bowel syndrome (Rome, 2006). These disorders can coexist with clear symptom overlap, which indicates that they may share common underlying pathophysiologies (Talley et al., 2003). A better understanding of colonic motility will undoubtedly enhance our understanding of some of these conditions.

1.9. RESEARCH AIMS

The aims of the studies performed within this thesis are:

1.9.1. GENERAL AIMS

1. to characterise colonic propulsive and motor activities in the human colon under basal physiological conditions;
2. to compare colonic motor activities in health and in slow transit constipation (STC) in an attempt to better characterise dysmotility in STC.

1.9.2 SPECIFIC AIMS

1. to determine the effect of recording methodologies on pancolonic motor activities. Specifically:
    a. to determine the impact, (if any) of prior bowel preparation
    b. to compare pancolonic manometric recordings obtained by two established methodologies: (i.e. water-perfused vs. solid-state technology).
2. to define the characteristics of propagating sequences throughout the entire colon of patients presenting with slow transit constipation and to compare these with those obtained from healthy volunteers.
3. to validate the use of new technology as a minimally invasive tool to measure colonic motility. Specifically:
    a. to validate the pH fall recorded by the wireless motility capsule around the ileocaecal valve as a marker use to determine colonic entry, and ultimately large bowel transit;
    b. to obtain normative values of colonic transit time and colonic contractile activities using the wireless motility capsule and compare these with those patients presenting with slow transit constipation.

2 RESEARCH METHODOLOGY

2.1. INTRODUCTION

Inclusion and exclusion criteria for healthy volunteers and patients suffering from slow transit constipation involved in clinical studies using colonic manometry and the wireless motility capsule (SmartPill Corporation, Buffalo, NY) are covered with this chapter. Specific methodologies used in certain clinical studies are discussed separately within each appropriate chapter.

2.2. ETHICS APPROVAL

Various research ethics committees were involved in the approval procedure for the clinical studies within this thesis. Furthermore, specific licences to handle radioactive materials during one of the studies were also obtained. For the main body of work contained within this thesis, the references for the ethics committees involved are as follows.

1. The Redbridge & Waltham Forest Local Research Ethics Committee (07/H0701/71) (Chapters 3, 4, and 5);
2. The Human Ethics Committees of the South Eastern Area Health Service, Sydney and the University of New South Wales (05/122) (Chapters 3 and 4);
3. The East London and The City Research Ethics Committee (07/H0703/77) (Chapter 6);
4. Certification from the Administration of the Radioactive Substances Advisory Committee (ARSAC) (reference number: RPC 564-935) (Chapter 6);
5. Approval from the Medicines and Healthcare Products Regulatory Agency (MHRA) obtained for using the wireless motility capsule (SmartPill) (Chapter 6) (reference number: CI/2008/0056);
6. Approval from University College of London Hospital (UCLH) (09/H0715/36) (Chapter 7).

2.3. RECRUITMENT AND SELECTION CRITERIA

Given that STC occurs almost exclusively in women, only females were recruited for most of the studies (exceptions are highlighted in specific chapters).

2.3.1. HEALTHY VOLUNTEERS

2.3.1.1. General inclusion criteria

Subjects included in these studies fulfilled all of the following:

1. aged between 18 - 75 years;

2. normal bowel movement (≥3 bowel movements per week) and ≤3 bowel movements per day;

3. no evidence or symptoms of evacuatory difficulties and/or regular abdominal pain as per symptom questionnaires filled during their screening visit;

4. body mass index < 35;

5. no current of planned pregnancy during the study period

2.3.1.2. General exclusion criteria for healthy volunteers

Subjects were excluded from studies if they had any of the following:

1. satisfied the Rome III criteria for irritable bowel syndrome (Rome, 2006);

2. significant medical and surgical history that may affect gastrointestinal function, except for uncomplicated appendicectomy, laparoscopic cholecystectomy and / Nissen fundoplication;

3. consumption of medications known to interfere with GI motility;

4. pregnant and lactating women.

2.3.1.3. Recruitment procedure

The recruitment of healthy controls involved in each study is explained in detail later within each specific chapter in the thesis.

2.3.2. STC PATIENTS

2.3.2.1. General inclusion criteria

Generally, patients involved in this research fulfilled all of the following:

1. satisfied the clinical criteria for idiopathic constipation as defined by the American College of Gastroenterology (Gastroenterology., 2005), and / or in whom the Cleveland Clinic constipation score (CCCS) was >15. The score range from 0 to a maximum score of 30; >15 was considered to represent at least moderate symptoms of constipation (Agachan et al., 1996).

The CCCS was developed in 1996 and consists of eight variables. In validation studies, all healthy volunteers scored less than 8. The score correlates well with the severity of constipation (Agachan et al., 1996) and shows a good sensitivity for detecting the response to treatment (Ortiz et al., 2012, Collins et al., 2012). However, the scoring system does not distinguish between physiological subtypes of constipation (Knowles et al., 2000);

2. passage of a 'complete' and satisfactory bowel movement on less than 3 days per week, for at least 2 of 3 weeks. These data were derived from 3 week stool diaries that detailed, on a daily basis, stool frequency and form and self-reported sense of complete evacuation (yes/no);

3. patients who failed to response to standard therapies including laxatives, dietary modification and exercise as documented by their clinicians;

4. normal colonoscopy within 5 years of enrolment (with the exception of colonic melanosis coli and non malignant colonic polyps);

5. Patients who had delayed colonic transit time, as confirmed by radio-opaque marker studies or colonic scintigraphy;

6. anorectal manometry showed normal anal sphincter function and no evidence of dyssynergic defaecation;

7. evacuation proctography showed normal rectal evacuation, with no functional (e.g. pelvic floor dyssynergia) or significant obstructive anatomical (e.g. functional

rectocoele > 2.5 cm in depth with retention of contrast, or occluding intussusception) abnormalities resulting in impediment to the expulsion of the radio-opaque contrast.

2.3.2.2. General exclusion criteria

The following patients were excluded from the research.

1. patients with significant concurrent medical illnesses, or those taking medications known to influence gastrointestinal motility and who were not able to stop their medications prior to and during the course of the study;
2. patients with previous history of gastrointestinal surgery (except for appendicectomy, cholecystectomy, and Nissen fundoplication);
3. women who were pregnant or lactating, or women of child-bearing age who were not on an acceptable method of contraception;
4. in studies involving exposure to radiation, subjects who had been exposed to other radiological or nuclear medicine investigations within 12 months prior to the investigations (except from simple ROM study) were excluded;
5. patients with previous history of gastrointestinal congenital abnormalities;
6. patients with previous history of inflammatory bowel disease or diverticular disease.

2.3.2.3 Recruitment procedure

The recruitment of STC patients involved studies included in this thesis is explained in detail within chapters 4 and 7.

2.4. DATA PROCESSING

2.4.1. DATA STORAGE

All data, regardless of method of acquisition, was stored on a dedicated password-protected computer within the named database, which was established at the start of the research and updated as required during the course of the research. All hard-copy data, including consent forms, questionnaires and data sheets, were stored in coded files within a secure cabinet in the Wingate Institute of Neurogastroenterology.

2.4.2 CLINICAL DATA

A full clinical history, including main presenting symptoms, mode of onset, past medical, surgical, obstetric and gynaecological events, medications, and family history, were derived from both information recorded in physiology reports and from self-reported questionnaires. Findings obtained from digital rectal examinations performed during patients' clinical visits were also recorded. STC patients had undergone proctoscopy, sigmoidoscopy, and/or colonoscopic examination prior to their referral to identify any underlying organic disease. These results were not included within the database.

2.5. RESEARCH TECHNIQUES USED WITHIN THIS THESIS

2.5.1. STANDARD LOWER GASTROINTESTINAL PHYSIOLOGICAL TESTING

Several routine clinical diagnostic tests were employed to assess colonic and anorectal function in the study cohorts. The ranges of normative values for these tests were derived from the GI Physiology Unit's control data [46 healthy volunteers for evacuation proctography (Palit et al., 2014) and 92 healthy volunteers for other tests of anorectal function (unpublished data)].

2.5.1.1. Rectal sensory testing

STC patients included in this research underwent simple volumetric rectal balloon inflation at 1 ml/s (Farthing and Lennard-jones, 1978) to assess rectal sensation, including first constant sensation, defaecatory desire volume (DDV), and maximum tolerable volume (MTV) as described previously in chapter 1 (1.4.5.1). Rectal sensation values gender stratified: MTV to balloon distension of <100 ml for men and <75 ml for women identified rectal *hyper*sensitivity, while MTV of >325 ml in men and >290 ml in women identified rectal *hypo*sensitivity. A single-use latex balloon attached to a 14-F Foley catheter was used for each study (Figure 2.01). Only patients with normal level of rectal sensation were recruited.

Figure 2.01. Equipment required to perform rectal balloon distension.

2.5.1.2. Anal sphincter morphology

Endo-anal ultrasound is a simple and well-established technique to demonstrate the integrity, thickness, and morphology of the internal and external anal sphincters were assessed using endo-anal ultrasound (Eckardt et al., 1994, Felt-Bersma and Cazemier, 2006). It is a central tool for the assessment of patients with faecal incontinence. In patient presenting primarily with constipation, assessment of anal sphincter integrity is also important to consider, given that constipation and significant faecal incontinence so frequently co-exist (Nurko and Scott, 2011). In addition, ultrasound can help in the diagnosis of underlying pathologies linked to constipation, such as full-thickness rectal intussusception, and solitary rectal ulcer, which are characterised by, increased thickness and displacement of the internal anal sphincter (Dvorkin et al., 2004). Additional findings such as a fistula truck, fluid collection, or other anomalies found during the examination were also recorded. However, only patients with no significant pathology were enrolled. Ultrasound was performed using a multi-frequency transducer (max. 16 MHz) (Figure 2.02), with two crystals placed back-to-back that rotated within the covering cone to produce a 360^{o} cross-sectional view of the muscle layers. The test provides clear structural details of the anal canal, however no information regarding anal sphincter function can be obtained. Normally, it is possible to identify four layers within the anal canal using endoanal ultrasound (Figure 2.03).

Figure 2.02. Endoanal ultrasound probe. The probe contains freeze-frame control in addition to two control points to move the transducer along the length of the anal canal. The probe is usually placed 4 - 5 cm proximal to the anal verge, at the level of puborectalis at the upper anal canal.

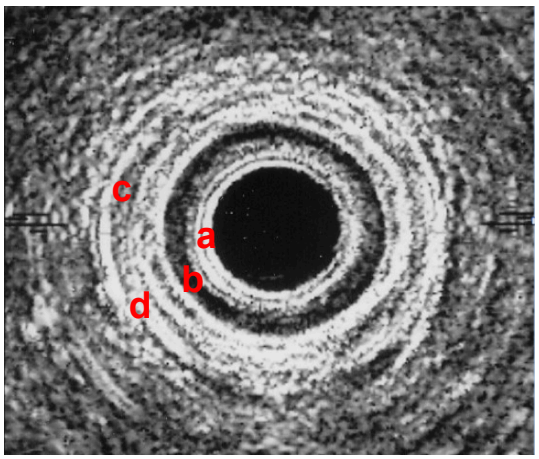


Figure 2.03. Normal structure of the anal canal. From internal to external, the separate layers are: (**a**) the submucosa, which appears as a highly hyperechoic (white) ring adjacent to the ultrasound probe, (**b**) the smooth muscle internal sphincter (IAS), which appears hypoechoic (black) and is approximately 2 mm thick, (**c**) the longitudinal muscle, which is a muscle band external to the IAS with similar echogenicity as the submucosa, and (**d**) the external sphincter (EAS), which appears as a modestly hyperechoic ring (Rottenberg and Williams, 2002), around 4-10 mm in thickness (Felt-Bersma and Cazemier, 2006).

2.5.1.3. Anal sphincter function

Station pull-through anorectal manometry was used to measure functional anal canal length, maximum resting tone, and maximum voluntary squeeze increments (Read et al., 1979, Rao et al., 2002). This was measured using a single sidehole water-perfused catheter (single-use) linked to a pneumohydraulic water perfusion system and pressure transducers. Pressure signals were transmitted to an amplification, recording and display system. The catheter was introduced through the anal canal and into the rectum. A period of recording stabilisation (usually 1 minute) was then performed to allow the recordings to stabilise. The pressure profiles were measured across a 5 cm distance proximal to the anal verge. Resting tone and squeeze increments were considered reduced if they were <50 cm H_2O.

2.5.1.4. Assessment of rectal evacuation

The balloon expulsion test and anorectal manometry are considered the basic tools to assess rectal evacuatory function (Remes-Troche and Rao, 2006). However, these tests do not exclude rectal structural obstructive abnormalities such as rectocoele which can be a cause of evacuatory dysfunction (Chapter 1, section 1.4.5.2). For subjects recruited to studies presented in this thesis, rectal evacuation was assessed using evacuation proctography (defaecography), a radiological examination in which instilled contrast is expelled under fluoroscopic control (Womack et al., 1985). The test was performed without prior bowel preparation and with the patient initially lying in the left lateral position with knees bent to the chest (Chan et al., 2001, Zarate et al., 2008). Barium sulphate contrast was instilled via a proctoscope (mixed with oats and water to a standard ratio to mimic stool consistency) using a calibrated large bore syringe to an amount sufficient to stimulate a sustained desire to defaecate (to a maximum of 600 ml). The patient was then transferred to a commode, where fluoroscopy was performed while they attempted to evacuate the neostool whilst sitting on a radiolucent commode. The procedure was terminated when the subject felt defaecation to be complete, or where they were unable to expel any more contrast (to a maximum times of 3 minutes).

Rectal evacuation is measured in terms of speed (time taken for evacuation) and effectiveness (percentage of contrast evacuated) (Dvorkin et al., 2005). The percentage of evacuated contrast was calculated from the difference between initial resting and post-evacuatory images, a modification of the technique that has been shown to correlate well with measured weights of evacuated contrast (Karlbom et al., 1999). Visual assessment of adequate opening of the anorectal angle and the anal canal, and the presence of any significant morphological abnormalities precluding or interfering with evacuation were also reported. At rest, and in health, the rectum is pulled forwards by the puborectalis, producing an angle of between 90 and 110^{o} between the rectum and anal canal (anorectal angle) to maintain continence (Lowry et al 2001). During the defaecation process (straining), the puborectalis muscle relaxes, allowing the anorectal angle to widen by at least 15^{o} (Lembo and Camilleri, 2003).

Functional rectocoeles that retain contrast were classified according to their depth: small (<2.5 cm), medium (2.5 - 4 cm), and large (>4 cm) (Shorvon et al., 1989, Siproudhis et al., 1992). Rectal intussusceptae were graded as described by Shorvon *et al.* (Shorvon et al., 1989) , with grades 4 - 7 (full-thickness circumferential intussusception) taken as significant (4 = recto-rectal; 5 = descent to the anorectal junction; 6 = recto-anal; 7 = overt prolapse). In addition to rectal obstructive structural abnormalities, proctography can also identify functional obstruction such as that seen with dyssynergic defaecation. This can be diagnosed when there is failure of the anal canal to open during defaecation, or failure of relaxation of the puborectalis, and hence opening of the anorectal angle (Scott and Gladman, 2008, Lunniss et al., 2009). The absence or poor expulsive forces can also be appreciated. Limitations of this test have previously been described in chapter 1 (section 1.4.5.2).

2.6.1.5. Colonic transit studies

whole gut or colonic transit times were assessed in patients using one of the following methods.

(a) A simple radio-opaque marker study (Hinton et al., 1969) was performed after the patient ingested a single gelatine capsule containing 50 markers (custom-made from radio-opaque tubing). A plain abdominopelvic x-ray was then taken at 100 h (Figure 2.04). A diagnosis of delayed colonic transit was made if the subject retained 20% or more of the markers (Roberts et al., 1993). Though this technique is a useful screening test for delayed transit, no individual patterns of transit delay can be accurately discriminated, which is the main limitation. Other limitations have been described previously in Chapter 1 (section 1.6.1.2.1).

Figure 2.04. Radio-opaque marker (ROM) study in a patient with slow transit constipation (Krogh and Christensen, 2009).

(**b**) Colonic scintigraphy was performed using a well-established method of oral administration of ^{111}I [DTPA] isotope, with consecutive gamma camera scans taken twice per day for 3 days (72) h (McLean et al., 1992, Roberts et al., 1993). Isotope retention was calculated from the entire colon on day 3 (with <9% isotope retention consider to be normal). However, only patients with severely delayed colonic transit (>50% isotope retention) were included.

2.5.2. PANCOLONIC MANOMETRY

2.5.2.1. Types of recording catheters

The recording of pancolonic contractile activities was achieved by using two types of catheters:

(1) a water-perfused manometric catheter, 4.5 m long and made of silicone (Dentsleeve, Wayville, SA, Australia) (Figure 2.05). The catheter contained 16 sidehole recording sites, spaced at 7.5 cm intervals from the tip; it was rendered radio-opaque due to a barium core and had an overall diameter of 3.5 mm.

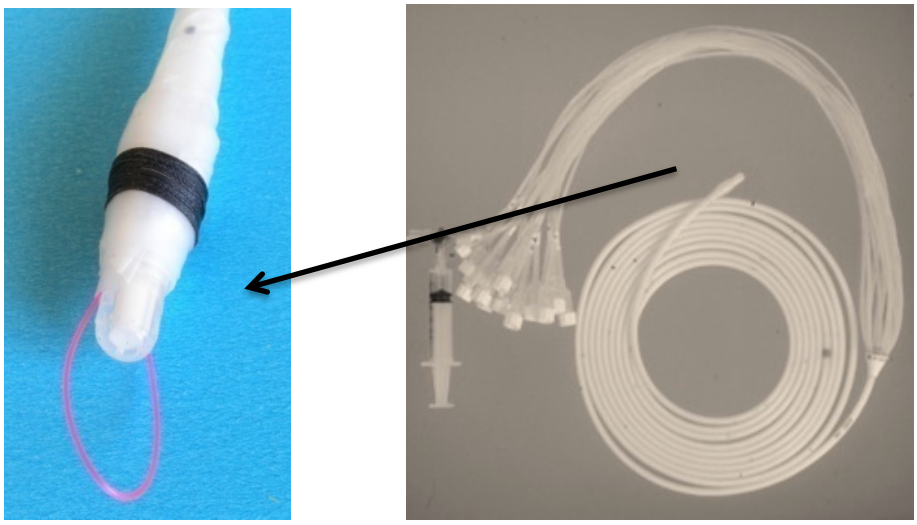


Figure 2.05. The water-perfused catheter with 16 ports spaced at 7.5 cm intervals from the tip, covered a total region of 112.5 cm (Dentsleeve, Wayville, SA, Australia). A thread attached to the catheter tip (left panel) was used to secure the catheter to the colonic mucosa via haemastatic clips.

(2) a solid state manometric catheter (UniTIP: Unisensor AG, Attikon, Switzerland), 2.55 m long with 20 pressure sensors spaced 7.5 cm apart (Figure 2.06) from the catheter tip. The overall diameter was also 3.5 mm. However, a maximum of 16 recording channels were introduced into the colon in each study. The number of channels that were outside of the colon were noted and removed from the study analysis. The catheter was calibrated using a long, custom-made airtight cylinder, to which low and high pressures were applied using a manometer (UniTIP: Unisensor AG, Attikon, Switzerland). The pressure value recording from each channel was confirmed as equivalent to the pressure applied by the connected manometer.

At the end of each study, catheters were cleaned, disinfected, and sterilised following sterilisation protocols as recommended by the manufacturer (see Appendix 5).

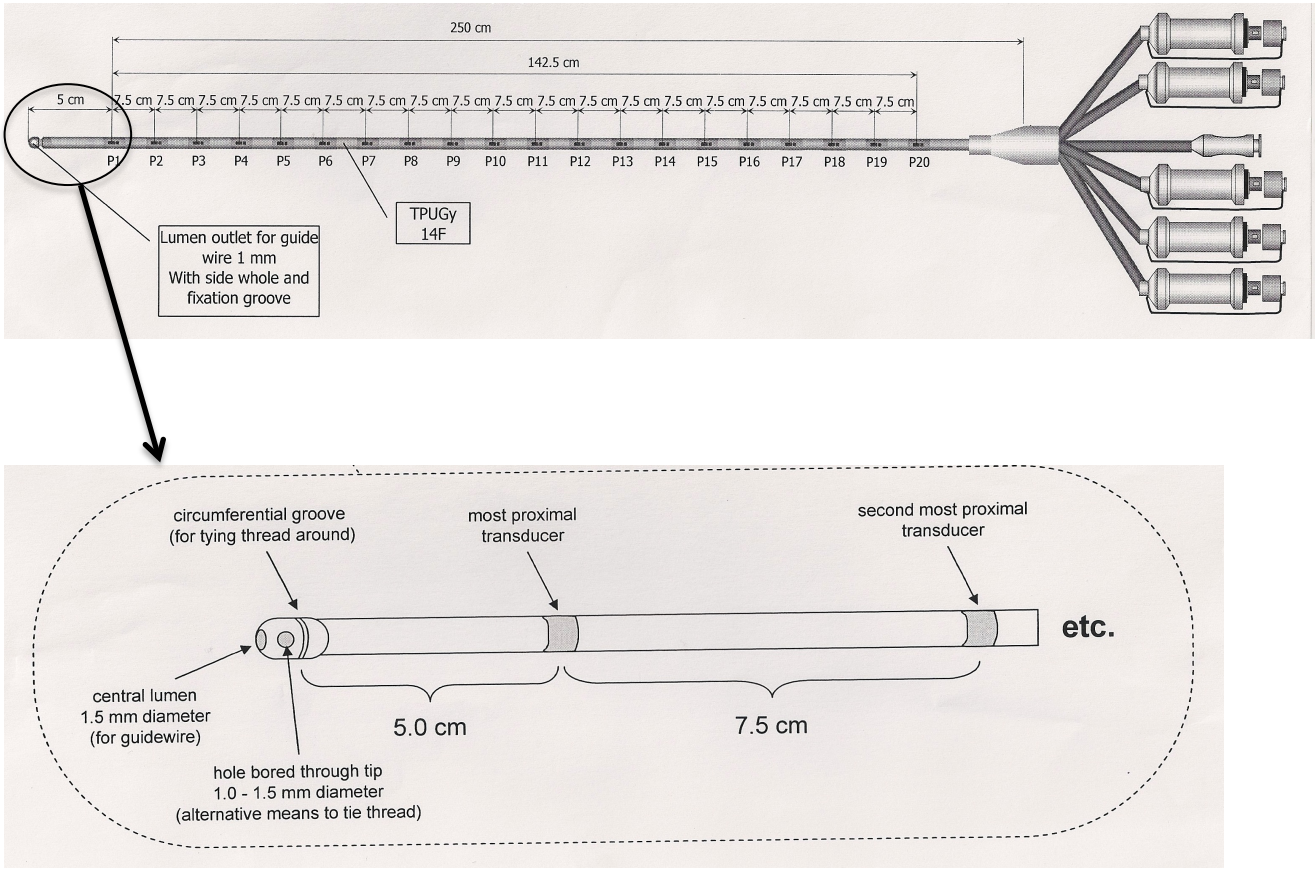


Figure 2.06. Design of the custom-made solid-state catheter incorporating 20 pressure transducers (UniTIP: Unisensor AG, Attikon, Switzerland). Intertransducer distance and catheter thickness was designed similar to the water-perfused catheters (Dentsleeve, Wayville, SA, Australia). The central lumen was not used for colonic intubation.

2.5.2.2. Colonic intubation

Colonic intubation was achieved using two methods:

(1) nasocolonic (antegrade) placement into the unprepared colon was achieved using water-perfused catheters (Dentsleeve, Wayville, SA, Australia) (Figure 2.05). A silicone balloon attached to the tip of the catheter could be inflated with water to facilitate transit through the small bowel and colon (Figure 2.07). The total duration of the intubation was up to 24 h until the catheter tip reached the desired location (i.e. at or beyond the sigmoid colon). Prior to the commencement of recording, the location of the catheter tip was confirmed fluoroscopically. The position of the catheter was checked again at the end of 24 h recording. Total fluoroscopy time was 30 - 90 seconds, and the maximum whole body effective radiation dose equivalent was 0.8 - 2.4 mSv.

(2) per-rectal (retrograde) catheter placement into the prepared colon was achieved with the aid of colonoscope using both water perfused (Dentsleeve, Wayville, SA, Australia) and solid-state catheters (UniTIP: Unisensor AG, Attikon, Switzerland) (Figure 2.05 and Figure 2.06 respectively). The day prior to intubation, subjects were given a clear fluid diet, and underwent colonic cleansing by oral administration of two bisacodyl tablets and polyethylene glycol (PEG) diluted in 4 litres of water, split into two doses (per standard practice of the Endoscopy Unit of The Royal London Hospital). Dividing the PEG dose significantly improves colon cleaning, increases patient compliance, and significantly decreases nausea as compared to one full dose (Di Palma and Rex, 2011).

On day 1, after an overnight fast and under conscious sedation with intravenous fentanyl, midazolam and hyoscine, the subject was asked to lie in a left lateral position to allow catheter intubation to the caecum under colonoscopic guidance.

The catheter was pulled in tandem to the colonoscope by a strong nylon loop tied to its tip and held in an endoscopic snare (Olympus America, Melville, NY, USA). Minimal air inflation was used, and air suction was performed at the time of extubation to reduce subject discomfort. Once the tip of the catheter reached the caecum (Figure 2.07 and Figure 2.08), the nylon loop on the catheter tip was

secured to a caecal fold using two hemoclips (Olympus America, Melville, NY, USA) (Figure 2.09). The colonoscope was then removed, leaving the catheter *in situ*. In most cases, confirmation of final catheter position within the colon following colonic intubation was obtained by 'freeze-frame' fluoroscopic assessment (Figure 2.08). This was important to confirm the site of the catheter tip, and to rule out looping of the catheter within the rectum, as this could cause a persistent urge to defaecate, erosion of the bowel mucosa, or damage to the catheter itself. The position of the catheter was checked again at the end of a 24 h recording. The total fluoroscopy time was <10 seconds, which equates to a maximum whole-body radiation dose equivalent of <0.4 mSv. Obtained images were used in localising pressure sensors for the purpose of data analysis.

Given the prolonged nature (up to 24 h) of pancolonic manometry studies, there is a well documented risk of catheter displacement (Rao et al., 2001b, Narducci et al., 1987, Bassotti et al., 1993b). Non-fixed catheters are likely to be expelled during defaecation, secondary to the force of propagating high amplitude contractions, and also consequent to recovery of colonic length following colonic intubation. Clinically, hemoclips are used endoscopically to close very deep intestinal ulceration, to clip large visible blood vessels to reduce the risk of perforation, and to stop bleeding from Mallory-Weiss tears (Hui and Sung, 2005). Rao *et al* examined the effect and the safety of endoscopic mucosal clipping of colonic manometric catheters during prolonged studies and showed that clipping significantly reduced catheter displacement and is safe to use (Rao et al., 2010b).

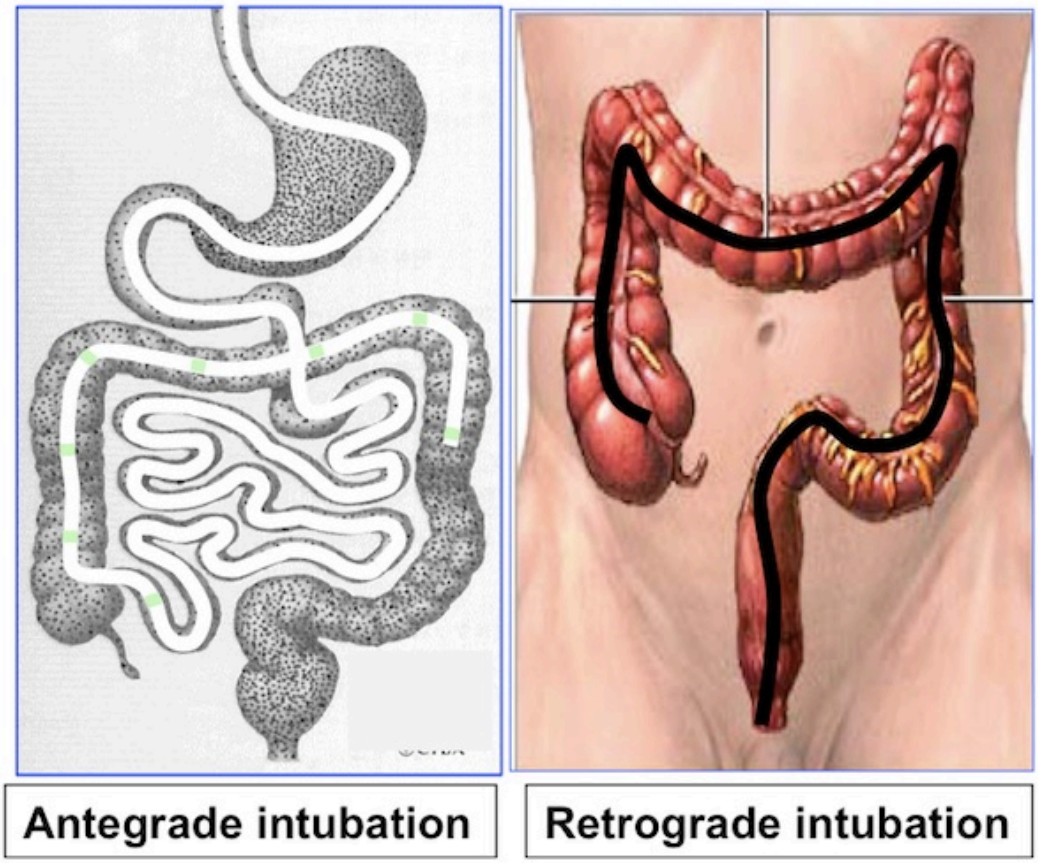


Figure 2.07. Methods of placement of pancolonic manometric catheters.

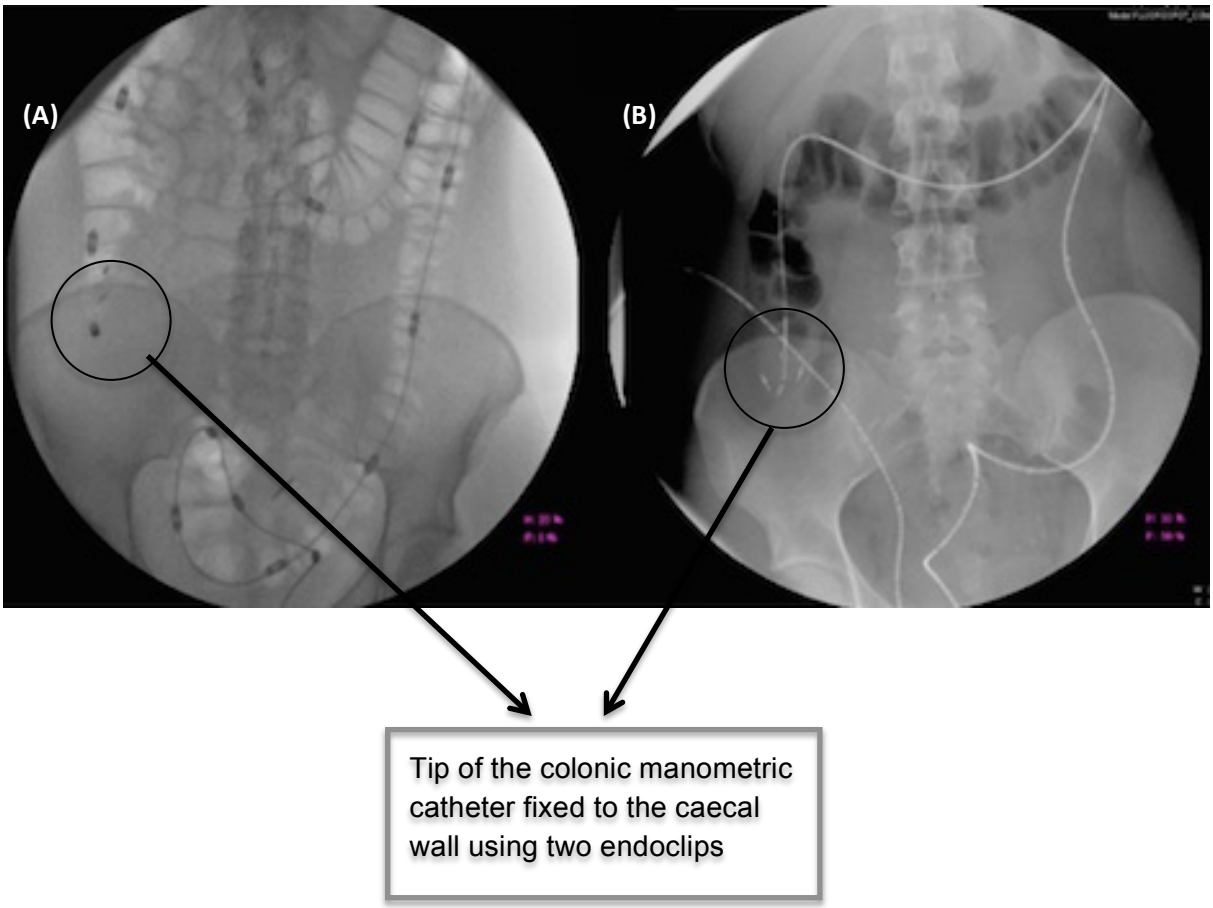


Figure 2.08. Freeze-frame fluoroscopic images for (A) solid-state manometry catheter and (B) water-perfused manometry catheter. Both catheters were placed via the per-rectal route into a prepared bowel using a colonoscope.

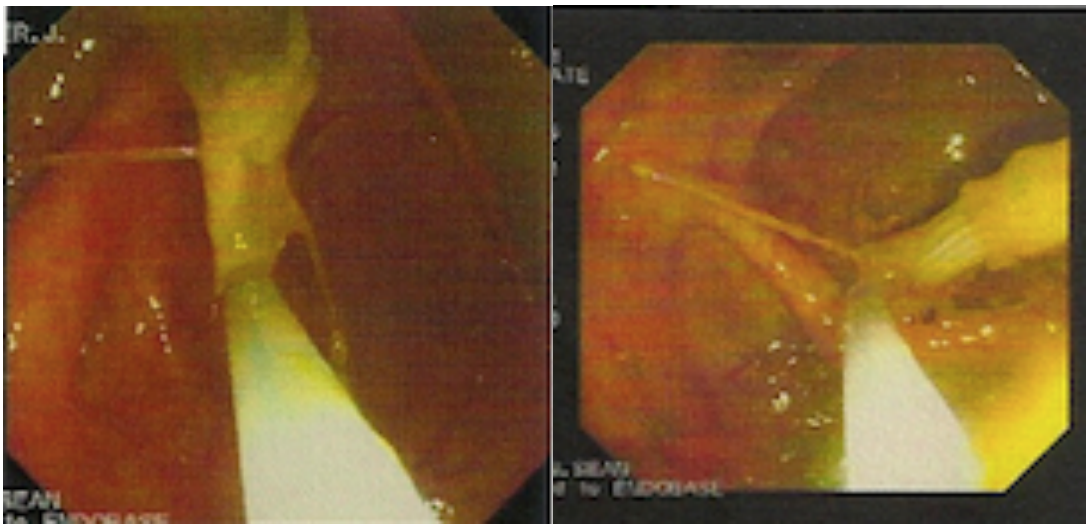


Figure 2.09. Colonoscopic images of the tip of the pancolonic manometric catheter within the caecum. A strong nylon thread attached to the tip is used for catheter fixation by endoclips.

2.5.2.3. Study Protocol

After recovery from sedation, subjects were transferred to a private room at the research centre (The Wingate Institute), where they slept overnight. Standard meals were given for lunch at 12:00 and for dinner at 18:00. Physical activity was minimised to reduce the risk of catheter detachment from the bowel lining.

Recording usually commenced at approximately 08:00 on day 2 (approximately 22 hours after intubation) to allow for washout of drugs and colonic re-filling, and was continued for 22 - 24 hours. All subjects were provided with the following standard meals during the recording period:

a. **Breakfast at 08:30 :** 500 Kcal (15% protein, 34% fat, 51% carbohydrate);

b. **Lunch at 12:00:** 1000 Kcal (24% protein, 43% fat and 33% carbohydrates);

c. **Dinner at 18:00:** 1000 Kcal (24% protein, 43% fat and 33% carbohydrates).

For water-perfused studies, the catheter lumen was continuously perfused with degassed, distilled water for the duration of the recording time (day 2 and 3) by use of a pneumohydraulic perfusion pump at a rate of 0.15 ml/min-^{1} (Dentsleeve).

Recordings were obtained using a customised modular manometric system (Solar Measurement System, software version 8.7b; Medical Measurement Systems, Enschede, the Netherlands) at the Royal London and using 16 external pressure transducers (Abbott Critical Care Systems, North Chicago, IL, USA), with recorded signals digitised at 10 Hz by preamplifiers (AqcKnowledge III Software, BIOPAC Systems, Inc., Santa Barbara, CA, USA) at the St George Hospital. All subjects were asked to press a marker to record any event including eating, sleeping and defaecation (if it occurred) and also the start and the end of the study.

For solid-state studies, the catheter was connected to a portable 20-channel solid-state recorder (Flexilog 3000, Oakfield Instruments Ltd., Oxon) with a large memory capacity (60 MB flash card, Sandisk) to enable recording for 24 hours. The recorder possessed an event marker and was secured in a harness worn by the subject. Again, all subjects were asked to depress the event marker to record any event that happened during the recording period.

For both methods, the recording period finished at around 09:00 hours on day 3, and colonic extubation was achieved by applying continuous but gentle traction as described by Fajardo *et al* (Fajardo et al., 2000).

2.5.2.4. Data analysis

For water-perfused manometric studies, each recording was exported directly from the MMS Solar recording system as a comma-separated values file (CSV) to a separate PC, where it was later converted to a text file and imported to specialised software (PlotHRM, Version1, Australia) for final analysis.

For solid-state manometric studies, the obtained recording was initially downloaded from the memory card within the recorder, to a portable computer using display software (Flexisoft III, Flexilog, Oakfield, England). The recorded data was then exported as a CSV file and converted to a text file to be uploaded to the PlotHRM analysis software.

For all studies, the text file was formatted, and pressure values recorded in each channel arranged within the text file according to channel location. Once a text file has been transferred to the PlotHRM software, the contractile activities within each channel appear as continuous linear tracings. The software has the ability to manually determine the direction of propagating activities and record them as values in a separate Excel spread sheet (Microsoft Corporation, Redmond, WA, USA), from which final statistical analyses are performed. This can be achieved by manually highlighting the propagative pressure waves, starting from the start channel to the last channel where the pressure wave terminated.

2.5.2.4.1. Definitions of propagating sequences

Due to the complexity of the data recording for the colon, only propagating sequences were considered during data analysis. Various parameters were analysed in healthy volunteers and compared to STC patients where appropriate. Analysis was conducted for both total and regional colonic motor activity (ascending, transverse, and descending colon). For the purpose of analysis and considering the number of pressure recording sideholes in recording catheters, the colon was divided into 16 regions (region 1 = caecum, region 4 = hepatic flexure, region 8 = splenic flexure, region 12 = proximal sigmoid colon, region 16 = rectum). Recording sideholes were assigned to the colonic region within which they lay as identified using fluoroscopic confirmation of the catheter position after colonic intubation and just before extubation.

The following steps were adopted to analyse PS:

1. identification: visual analysis of the manometric trace was used to identify the PS. They were defined as an array of three or more pressure waves recorded from adjacent recording sites in which the conduction velocity between wave onset was between 0.2 and 12 cms^{-1} (Bampton et al., 2000, Bampton et al., 2001);

2. the peak of each manually identified pressure contractions (as part of PS) was provided automatically by the analysis software as a numerical value;

3. duration of each PS was also automatically calculated by the analysis software;

4. polarity: PS were qualified by the terms antegrade or retrograde, depending upon

their polarity (direction) of propagation;

5. amplitude: PS were classified as a high amplitude PS (HAPS) if the amplitude of at least one component propagating pressure wave was >116 mmHg (Bampton et al., 2001). This is based on values derived from normal mid-colonic mean amplitude + 2SD recorded in the healthy unprepared colon (Bampton et al., 2000, Bampton et al., 2001). In the first stage of analysis, all PS and HAPS were grouped based upon polarity (i.e. antegrade or retrograde). In secondary analysis, HAPS were removed from the data set and dealt with as a separate entity. Other PS, including all pressure waves of <116 mmHg were considered as low amplitude PS (LAPS);

6. PS and colonic response to meals: the influence of feeding on colonic motor activity, the so-called *gastro-colonic response*, was evaluated by providing standardised meals (index meal =1000 kcal given at lunch time) to each subject. The 2 h epoch prior to and after the meal was divided into four 30 min periods. In each of these periods, the HAPS frequency and the retrograde and antegrade PS frequency, velocity, amplitude and extent of propagation were detailed. The study of the gastro-colonic response is fundamental, as previous studies have suggested that this response may be altered in patients with constipation (Kamm et al., 1988, Bassotti et al., 1992b, De Schryver et al., 2003);

7. characteristics of PS and diurnal variation: the PS frequency per hour in the 8 h epoch between 22:00 and 06:00 hours (nocturnal period) was compared to the frequency per hour between 14:00 - 22:00 and 06:00 - 14:00. Amplitude of PS was also evaluated during daytime and nocturnal period;

8. defaecation and PS spatiotemporal organisation: predefaecatory PS were defined as present and normal if within the 20 min period prior to stool expulsion, three or more PS were identified. These final three PS normally display a distal to proximal colonic regional shift in the site of origin leading up to defaecation (Bampton et al., 2000). The association between this stereotypic pattern and episodes of defaecation was examined in data obtained from both recording systems in healthy volunteers and from STC patients. The frequency of defaecation and stool form was also recorded for both during all studies;

9. Spatiotemporal organisation and regional linkage of antegrade and retrograde PS: global appreciation of spatiotemporal patterning of PS of 24 hour pan-colonic recordings was assessed by means of spatiotemporal pressure mapping in a condensed format. This method has been recently validated to permit an overall view of colonic antegrade and retrograde colonic PS for 24 hours within a single figure (Dinning et al., 2009b, Dinning et al., 2008b) (Figure 2.10). In addition, it allows better appreciation of patterns of colonic contractile activities, especially when comparison has to be made between healthy controls and patients. Spatiotemporal mapping was performed in collaboration with Dr Phil Dinning. A PS was deemed regionally linked to the PS immediately preceding it, if the two PS originated from different colonic regions but the segments of colon traversed by each PS overlapped. The regional linkage was only assessed between sequential antegrade PS as retrograde PS are not reported to have any spatiotemporal organisation (Dinning et al., 2009b, Dinning et al., 2008b).

In summary, antegrade and retrograde PS that had been previously identified and logged within the spreadsheet were used to create a spatiotemporal map for each manometric study. Within each spreadsheet, each row represented a 30-second time interval (2880 rows = 24 h), and each column represented an individual colonic region, within which an individual recording channel was allocated (Dinning et al., 2008b). Each individual PS was logged in a row as a numerical value, equivalent to the time at which it occurred. The numerical value in each cell represented the amplitude of the pressure wave.

Interpolation of empty cells, where no recording could be obtained from the corresponding recording channel due to loss of signal for short periods were added. This is performed by populating the last recorded pressure value into all empty cells. In fact, most of the missing recording period was due to the fact that the recording catheter was unable to record pressure waves exceeding 330 mmHg (Chapter 5, Figure 5.03). The numerical value of each retrograde PS was then multiplied by (-1) to get a negative value. The final Excel spreadsheet was then transferred to multipurpose data algorithm development and visualisation software (MATLAB 7, Mathworks, Natick, MA, USA), with the ability to create a colour map with gradient colors to represent the direction of each PS; a green colour gradient depicted antegrade PS (to all positive pressure values), and a red colour gradient depicted

retrograde PS (to all negative pressure values). The gradient variation within each colour represents the PS amplitude; the higher the amplitude, the darker the colour on the map. The three-dimensional spatiotemporal map represented the three sets of data: time of day on the y-axis, colonic regions on the x-axis, and amplitude of each PS on the z-axis (Figure 2.10) (Dinning et al., 2008b).

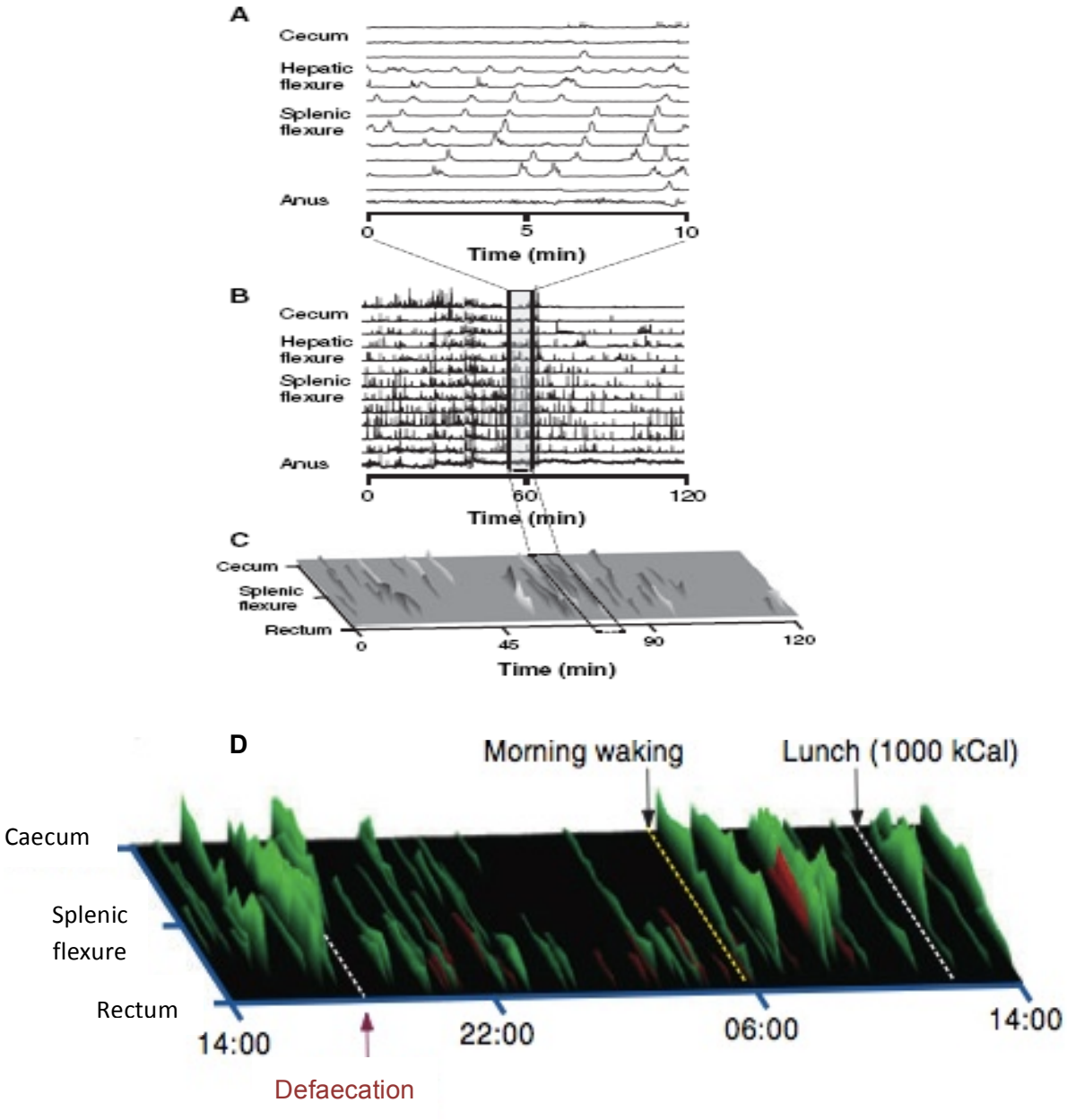


Figure 2.10. Development of a spatiotemporal map for colonic propagating sequences (PS) over a 24 h recording. (**A**) shows a segment of a manometric trace recorded in a patient with obstructed defaecation; (**B**) demonstrates that by compressing the trace to gain an impression of the PS activity over a 2 h period, the ability to identify individual PS is lost; (**C**) The same 2 h section of trace displayed in (B) is reproduced as a spatiotemporal map. Within this map, each individual ridge represents a PS. Antegrade PS (grey to white) originate at the orad end of the ridge, and retrograde PS (dark grey to black) originate at the anal end of the retrograde ridge. The start of each antegrade and retrograde ridge indicates the site of origin and the time of day the PS occurred. The length of the ridge indicates the extent of propagation. The shading within the ridge indicates the amplitude of the component pressure waves; (**D**) example of the standard twenty-four hour colour spatiotemporal maps showing antegrade (green) and retrograde (red) colonic propagating sequences in healthy female. [Adopted from (Dinning et al., 2008b)].

2.5.3. WIRELESS MOTILITY CAPSULE (SMARTPILL)

The study protocol for wireless motility capsule studies are described in detail within their specific chapters (Chapters 6 and 7).

2.6. LITERATURE REVIEW AND REFERENCING

References within this thesis were obtained by literature review performed during the research period (2009 to 2014), using the Medline search from Pubmed, and National Centre for Biotechnology Information [http://www.ncbi.nlm.nih.gov/pubmed]. Only studies and reviews in the English language were reviewed and used within this thesis. All references have been prepared in accordance with the uniform requirements for manuscripts submitted to medical journals and developed by the international Committee of Medical Journal Editors (*N Engl J Med* 1991; **324**: 424-428), based on formats for bibliographical references first developed by the Vancouver Group.

3 PANCOLONIC MOTOR FUNCTION IN HEALTH: INFLUENCE OF RECORDING TECHNIQUE

3.1. INTRODUCTION

Assessment of pancolonic motor function is fundamental both to our understanding of the physiology of the large bowel (Scott, 2003), and may also be important in helping to elucidate the pathophysiological mechanisms underlying disorders such as slow transit constipation (STC). As discussed previously in chapter 1, there is a lack of standardisation with regard to the technique used to assess colonic motor function, which is considered a principal limitation. Colonic manometric catheters can be intubated by a retrograde (per-rectal) or antegrade (per-nasal) approach, or even a combination of both (Scott, 2003, Dinning et al., 2009a, Dinning et al., 2010). Nasocolonic (antegrade) catheter placement into the unprepared colon is appealing, as it provides colonic motility recordings in conditions closer to the true physiological state with stool *in situ*. However, antegrade placement requires sufficient peristaltic propulsion to progress the catheter tip into the desired colonic region, and while this technique is feasible in healthy controls and in patients with relatively normal transit (Dinning et al., 2004), the technique may not be suitable for use in patients with severe colonic dysmotility (e.g. STC). Consequently almost all colonic manometric studies performed in patients with bowel disorders have used retrograde catheter placement. In addition, given that there is a lack of normative data defining colonic motility in health, there is a need to use a more cost- and time-effective manometric technique, to enable us to perform more studies in healthy volunteers and in patients with suspected colonic dysmotility in wider clinical studies. However, one crucial issue to understand is whether prior bowel cleansing influences any of the manometric measures that might represent pathological markers for dysmotility (and specifically for STC for the purpose of this research). Clarification of the impact of bowel preparation, if any, on colonic motor activity is also fundamental to permit comparison among studies adopting different manometric techniques.

To date, the effect of bowel cleansing upon colonic contractile activity remains unclear. Dinoso *et al*. reported no apparent change with bowel preparation in the distal colon (Dinoso et al., 1983). Conversely, Lemann *et al*., who recorded motor activity over 60 cm from the ascending colon, demonstrated an increase in frequency of high amplitude propagating contractions or sequences (HAPS), in the prepared colon of healthy volunteers (Lemann et al., 1995). This supports previous work

performed in an animal model by Sarna, who showed an increase in the frequency of giant migrating contractions (canine counterpart to human HAPS), in the cleansed bowel of strain-gauge instrumented dogs (Sarna, 1992). In other animal studies the effect of whole gut irrigation on small and large bowel smooth muscle activities was determined as insignificant (Soyer et al., 2009). However, no studies, to date, have been able to clearly demonstrate the effect of bowel preparation on true pan-colonic motility in humans. This is very important for comparing and interpreting colonic manometric studies, and also important if retrograde colonic intubation is to be used in wider studies (both research and clinical purposes), where prior bowel preparation is always required.

3.2. STUDY AIM:

To determine the impact (if any) of prior bowel preparation on colonic contractile activities in the healthy human colon, specifically: the characteristics of PS, spatiotemporal organisation, colonic meal response, and stereotypic predefaecatory motor patterns.

3.3. MATERIALS AND METHODS

3.3.1. STUDY POPULATION

3.3.1.1. Healthy volunteers:

Recruitment was from two sites:

(1) UK studies: nine healthy volunteers [all females; median age 34 (range: 24 - 56)] were studied using colonoscopic-assisted placement of a pancolonic water-perfused catheters into the *prepared* bowel. They were identified through advertisement placed on the Queen Mary University of London website in and circulated amongst College webmail users. Initially, healthy volunteers invited to take part the study attended a screening and direct interview. Prior to this visit, they were asked to complete a set of health assessment questionnaires, which included demographic data, past medical and surgical histories, a validated quality of life questionnaire (SF-36) (Ware and Sherbourne, 1992, McHorney et al., 1993, Ware, 2000), Bristol stool

score (Lewis and Heaton, 1997), and a stool diary (see appendices 2 - 4). During the screening visit, appropriate consent was also obtained.

(2) Australian studies: 8 healthy controls [two males; median: 26 (range: 22 - 47)] were studied using nasocolonic intubation of a similar catheter into the *unprepared* bowel. These studies were performed in Australia in collaboration with Dr Phil Dinning and Professor Ian Cook. Subjects were identified through an advertisement.

All healthy subjects however, satisfied the general inclusion criteria as previously explained (Chapter 2, (2.3.1)). In summary, they all had a normal bowel habit, defined as between three bowel movements a day and one bowel movement every 3 days, with no symptoms of rectal evacuatory difficulty or the irritable bowel syndrome, as defined by Rome III criteria (Rome, 2006). None were taking regular medications including laxatives, and none had a history of prior abdominal surgery, other than those stated in the general inclusion criteria.

3.3.2. COLONIC MANOMETRIC TECHNIQUES

See Chapter 2, section 2.5.2.

3.3.3. DATA ANALYSES AND PRESENTATION

See Chapter 2 section 2.5.2.4.

3.4. STATISTICAL ANALYSIS

A Mann-Whitney U test was used to examine direct comparisons between all PS, low amplitude PS, and HAPS characteristics (frequency, amplitude, velocity, site of origin and extent of propagation), between the prepared and unprepared colon groups. The comparisons between these variables were made for the total colon, for the right colon (ascending and transverse colon) and for the left colon (descending and sigmoid colon). The same test was also used to compare regional linkage that existed between the two control groups. Comparisons between basal and postprandial PS characteristics within subjects was performed using a paired *t* test. Comparisons between the delta values (basal - postprandial) between the prepared and unprepared groups were performed using the Mann-Whitney U test.

Chi-squared analysis was used to compare the number of episodes of stool expulsion associated with the stereotypic predefaecatory pattern of PS in each

group. Where appropriate, the coefficient of variation (CV) was provided to give an indication of variability that exists within certain measured parameters. Data are expressed as mean ± SD, apart from frequency of defaecation, which is expressed as median and range. A *P* value < 0.05 was considered statistically significant.

3.5. RESULTS

Sixteen subjects (8 healthy controls from the UK and 8 healthy controls from Australia) completed the study without complication and catheter position was maintained in all. One subject from the UK site who underwent colonoscopic catheter placement into the prepared colon experienced premature catheter displacement following defaecation, and therefore, was excluded from subsequent data analysis.

3.5.1. ANTEGRADE PROPAGATING SEQUENCES

Overall, the mean amplitude of PS was significantly increased in the prepared colon [70 ± 13 mmHg (CV: 19%) vs. 46 ± 10 (CV: 22%) mmHg; *P* = 0.004, (Table 3.01, Figure 3.01).

The total and regional colonic frequency, velocity and site of origin and extent of propagation of PS did not differ between the prepared and unprepared bowel (Table 3.01, Figure 3.01). In both groups, PS originated with a significantly greater frequency in the right than left colon (*P*< 0.001, Table 3.01, Figure 3.01). The extent of propagation of PS originating in the left colon of both groups was significantly greater than the extent of propagation of PS originating in the right colon (Table 3.01; Figure 3.02).

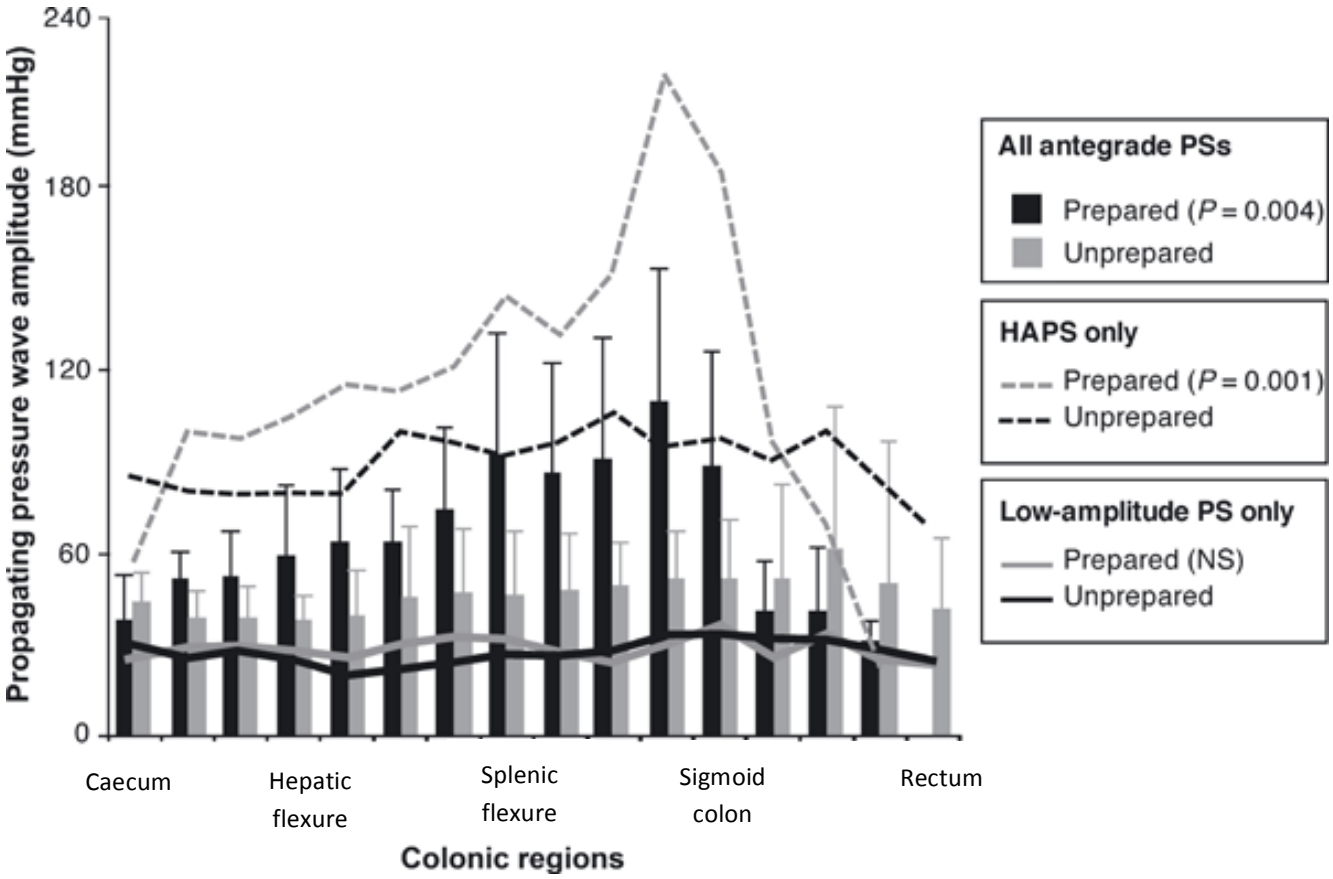


Figure 3.01. Regional variation in the amplitude of antegrade propagating sequences (PS), high amplitude propagating sequence (HAPS) and low-amplitude propagating sequences. The histogram shows the amplitude of all propagated pressure waves identified at each colonic region. In the prepared colon, the amplitude of propagating pressure waves is significantly increased (P = 0.004). The hatched lines indicated the amplitude of component pressure waves in the HAPS. The amplitude of the HAPS is also significantly increased (P = 0.001) in the prepared colon. The solid lines represent the amplitude of all of the remaining PS (low-amplitude PS), once the HAPS have been removed from the data set. Note there is no difference in the amplitude of low-amplitude PS between the two groups.

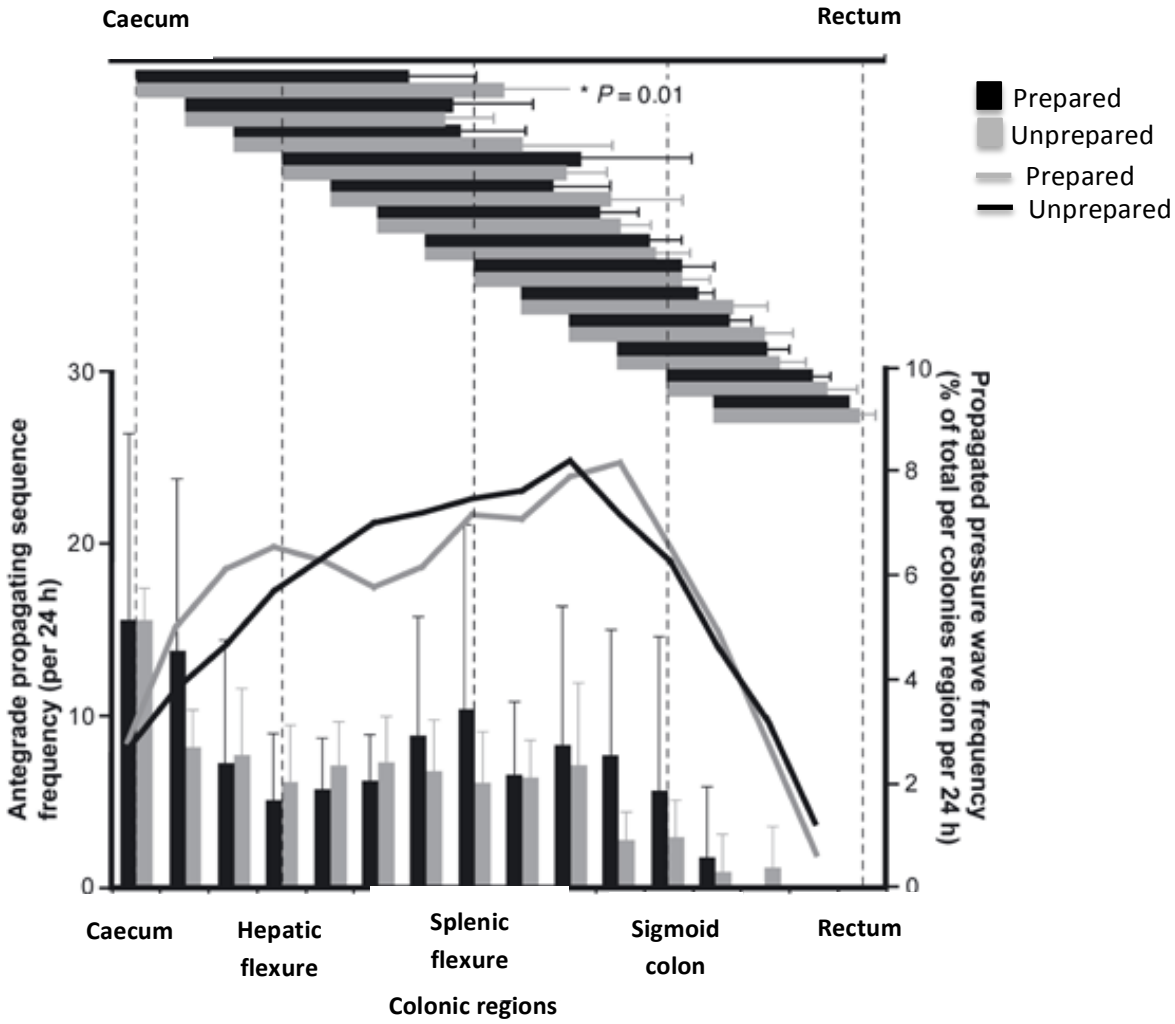


Figure 3.02. Regional variation in the frequency of initiation and extent of propagation of antegrade propagating sequences (PS). The histogram at the bottom shows the distribution of antegrade PS grouped according to the site of origin. The horizontal bars at the top show the mean extent of propagation by sequences originating at the same site. Note that in both groups, PS originate significantly (P< 0.001) more frequently in the proximal than in the distal colon. The extent of propagation of PS is greater for sequences originating in the proximal colon in both groups. The extent of propagation differs between groups in the caecum only. PS originating in the caecum of the unprepared colon extend further (P = 0.01) than those originating in the same region in the prepared colon. The solid lines are proportional to the propagating sequence frequency (histograms shown at the bottom), and indicate that the density of component pressure waves is highest between the splenic flexure and distal descending colon and lowest at the extremities of the colon.

		Prepared colon			Unprepared colon		
		Right Colon	**Left Colon**	**Total Colon**	**Right Colon**	**Left Colon**	**Total Colon**
Antegrade PS	**Frequency (per 24h)**	58 ±34*	38 ± 30	96 ± 60	40 ± 9*	21 ± 10	62 ± 7
	Velocity (cm/s)	1.5 ± 0.5	1.7 ± 0.4	1.6 ± 0.4	1.5 ± 0.3	1.4 ± 0.4	1.4 ± 0.3
	Amplitude (mmHg)	62 ± 14#*	78 ± 22#	70 ± 13#	47 ± 18	47 ± 25	46 ± 10
	Extent of propagation (cm)	34 ± 6*	21 ± 2	29 ± 5	40 ± 6*	24 ± 4	33 ± 5
Retrograde PS	**Frequency (per 24h)**	21 ± 34	22 ± 26	43 ± 57	5 ± 5	12 ± 11	17 ± 10
	Velocity (cm/s)	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.3	1.5 ± 0.6	2.5 ± 1.8	1.9 ± 1.2
	Amplitude (mmHg)	29 ± 5	34 ± 8	32 ± 6	26 ± 9	24 ± 6	25 ± 6
	Extent of propagation (cm)	18 ± 3	20 ± 3	19 ± 3	26 ± 5	22 ± 6	22 ± 5

Table 3.01. Antegrade and retrograde propagating sequence characteristics (PS). For ease of presentation *P* values between 0.002 and 0.01 are represented as *P*< 0.01. All other *P* values are represented as *P*< 0.001. * A significant difference (*P*< 0.01) between the left and right colon within subjects. + A significant difference (*P*< 0.01) between antegrade and retrograde characteristics within subjects for the same region. # A significant difference (*P*< 0.01) between subject groups for the same region.

3.5.2. RETROGRADE PROPAGATING SEQUENCES

The retrograde PS frequency, velocity, amplitude and site of origin and extent of propagation did not differ between the two groups (Table 3.01).

3.5.3. HIGH AMPLITUDE PROPAGATING SEQUENCES

In the prepared colon group, there was a significant 2.5 fold overall increase in HAPS frequency [22 ± 7 (CV: 33%) vs. 8 ± 4 (CV: 50%) HAPS/24 h; *P* = 0.003, Table 3.02], and HAPS overall were also of a significantly greater amplitude [126 ± 20 mmHg (CV: 16%) vs. 90 ± 17 (CV: 19%) mmHg; *P* = 0.001: Table 3.02, Figure 3.01].

Notably, the extent of propagation of HAPS originating in the ascending colon was significantly reduced in the prepared bowel (*P* = 0.005; Table 3.02). In both groups, 44% of HAPS originated in the ascending colon, and >75% originated proximal to the splenic flexure.

		Prepared colon			Unprepared colon		
		Right Colon	**Left Colon**	**Total Colon**	**Right Colon**	**Left Colon**	**Total Colon**
HAPS	**Frequency (per 24h)**	19 ± 5*#	3 ± 2#	22 ± 7#	6 ± 4	2 ± 2	8 ± 4
	Velocity (cm/s)	1.1 ± 0.2*	1.5 ± 0.3	1.3 ± 0.3	0.8 ± 0.3	1.3 ± 0.6	1.1 ± 0.3
	Amplitude (mmHg)	110 ± 20*#	153 ± 26#	126 ± 20#	83 ± 23	94 ± 24	90 ± 17
	Extent of propagation (cm)	47 ± 8*#	24 ± 3	38 ± 6	65 ± 13*	28 ± 8	50 ± 15

Table 3.02. High amplitude propagating sequence characteristics in the prepared and unprepared colon (HAPS). For ease of presentation *P* values between 0.002 and 0.01 are represented as *P*< 0.01. * A significant difference (*P*< 0.01) between the left and right colon within subjects. # A significant difference (*P*< 0.01) between subject groups for the same region.

3.5.4. LOW AMPLITUDE PROPAGATING SEQUENCES

There were no differences with regard to frequency, amplitude, velocity or extent of propagation of low-amplitude PS between the two groups (Figure 3.01).

4.5.5. COLONIC MEAL RESPONSE

In both groups, a 1000 kcal meal induced a significant increase in HAPS frequency compared to the basal period immediately preceding it (prepared: 1 ± 1 vs. 4 ± 2; P = 0.01 per h; unprepared: 0.3 ± 0.7 vs. 2 ± 1 per h; P = 0.005). Comparison of the delta values (HAPS basal - HAPS postprandial) showed no difference between the two groups, indicating that the meal response was similar between the groups. The increase in HAPS was not specific to any particular 30 or 60 min epoch postprandially. The meal had no effect upon low amplitude PS characteristics in either group.

3.5.6. DIURNAL VARIATION IN PROPAGATING SEQUENCE FREQUENCY

In both groups, there was a two-fold decrease in the PS frequency during the nocturnal period (prepared: 5 ± 3 vs. 2 ± 2 PS/h, P = 0.03; unprepared: 3 ± 1 vs. 1 ± 1 PS/h, P = 0.01). A comparison of the delta values (day frequency - nocturnal frequency) indicated no significant difference between the groups.

3.5.7. SPATIOTEMPORAL ORGANISATION OF ANTEGRADE AND RETROGRADE PROPAGATING SEQUENCES

The proportion of antegrade PS that showed spatiotemporal organisation was significantly reduced in the prepared colon. Of all antegrade PS, 82 ± 9% were regionally linked in the unprepared bowel, compared to only 57 ± 9% (P< 0.001) in the prepared colon.

3.5.8. DEFAECATION AND SPATIOTEMPORAL ORGANISATION OF PREDEFAECATORY PS

Defaecation frequency was significantly increased in the prepared bowel compared to the unprepared (2.8 ± 0.7 vs. 1.2 ± 0.5 bowel motions/24 h; *P* = 0.006). Overall, 21 episodes of defaecation, of which mostly watery stool was expelled, were recorded in seven subjects with a prepared bowel (one volunteer was excluded from defaecation analysis, as it was unclear from their diary entries if they had opened their bowels or simply urinated), compared to 10 episodes of defaecation in the unprepared bowel group. Stool consistency in the unprepared bowel did maintain some form, although was generally described as very soft. Interestingly, none of the volunteers in the prepared colon group defaecated during the first 22 h after placement of the catheter when no water infusion occurred. Overall, PS were associated with all but one episode of defaecation (in a subject who had undergone bowel cleansing), with 60% classified as HAPS in the prepared bowel group, and 63% in the unprepared bowel group (*P* = NS). In addition, the number of HAPS or PS associated with each bowel movement was similar between both groups. However, the stereotypic pattern of PS prior to stool expulsion was only evident in 5 of the 21 episodes (23%) of defaecation in the prepared group, compared to 9 of 10 episodes (90%) in the unprepared group (*P* = 0.001; Figure 3.03).

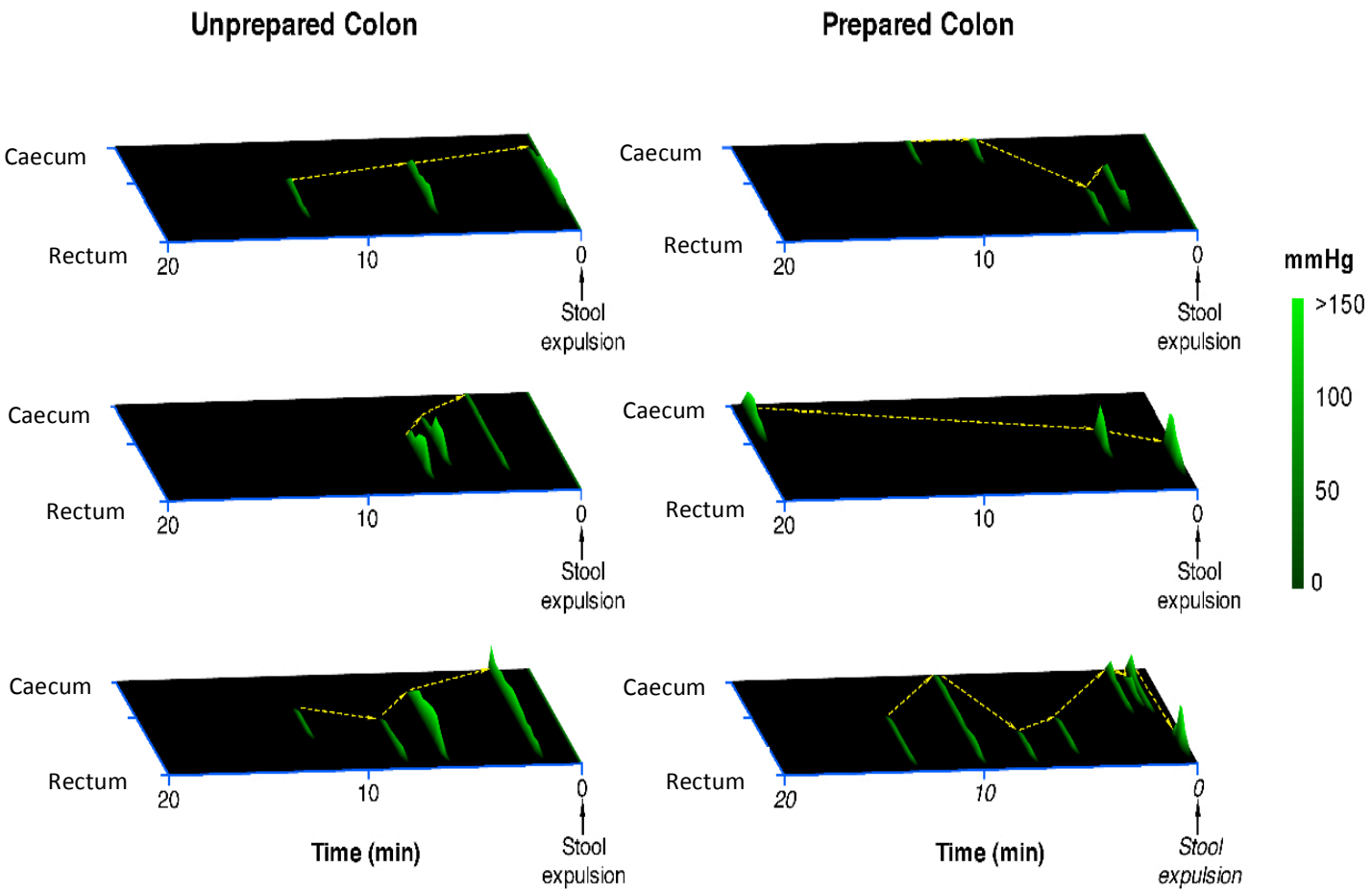


Figure 3.03. Spatiotemporal maps of colonic propagating sequences (PS) in the 20 minutes period prior to stool expulsion in three subjects within the prepared and unprepared groups. In every map, each individual ridge represents an antegrade PS. The start of each ridge indicates the site of origin and the time of day the PS occurred. The length of the ridge indicates the extent of propagation. The shading within the ridge indicates the amplitude of the component pressure waves. The hatched arrows link the site of origin of sequential PS. In the unprepared colon there is a stereotypic distal to proximal shift in the site of origin of the final three PS prior to stool expulsion. Note that this pattern is not evident in the prepared colon.

3.6. DISCUSSION

To date, the vast majority of colonic manometric studies available in the literature have adopted colonoscopically-assisted catheter placement that requires appropriate bowel preparation to provide better visibility during colonic intubation. Nasocolonic intubation does not require the bowel preparation to be prepared, and can provide a recording of colonic motility in conditions near to physiological status, where the bowel is filled with chyme; although this method is less appealing because it is more difficult to achieve and is practically challenging. However, the effect of bowel cleansing on pancolonic motility (specifically, colonic motor activities) has never formally been investigated.

By comparing pancolonic manometric recordings obtained from both the prepared and unprepared colon using similar catheter assemblies, the present study has shown that prior bowel preparation influences the characteristics of some parameters. Nevertheless, a number of fundamentally important and reproducible colonic motor responses, in which PS feature prominently, as well as overall PS frequency, appear not to be influenced by bowel preparation. In this study, we did not observe any significant change in the overall PS characteristics (frequency, extent, polarity and velocity), or in the response to physiological stimuli such as augmentation of PS following the meal, augmentation of PS following morning waking, and nocturnal suppression of PS activities. These findings allow investigators from different laboratories to make valid comparisons between colonic manometric data derived from the prepared and unprepared colon, although there are some caveats. Specifically, if HAPS frequency, PS amplitude, the regional linkage among consecutive PS, and also predefaecatory stereotypical patterning are of primary interest in a specific study, then measures must be controlled and compared with normative datasets derived from comparable catheters and experimental conditions (i.e. presence or absence of bowel preparation).

Some studies have assessed the effect of prior bowel preparation on colonic transit time (CTT) measured by radio-opaque markers (ROM) as an indirect marker of

colonic motor activities. A recent study in paediatric patients showed that the presence of colonic content significantly influenced CTT measurement, as CTT appeared to be significantly decreased (i.e. faster) in a previously cleaned colon than a faecally loaded colon (Quitadamo et al., 2015). Another study in adult constipated patients reports similar findings (Sloots and Felt-Bersma, 2002). Previous colonic manometric studies, performed in both humans and dogs have shown similar findings to our present study, with bowel cleansing reported to induce an increase in the frequency of HAPS (Lemann et al., 1995, Sarna, 1992). Unlike earlier reports however, we further showed an increase in the amplitude of HAPS (Lemann et al., 1995, Sarna, 1992). One possible explanation might be that the presence of thick intraluminal bowel content has a 'damping' effect, and thereby the magnitude of the recorded pressure wave is lower in the unprepared colon. This assumption would be particularly relevant in the left colon, where more viscous and semi-solid stool consistency is normally present, and where in the prepared bowel we recorded the greatest increase in the amplitude of PS. Given the fact that HAPS are defined exceeding a certain amplitude cut-off, and there was an overall increase in pressure waves amplitude following bowel preparation, it is not surprising that the frequency of HAPS are also increased. The criteria for defining HAPS as adopted in this study, states that PS need at least one of their continuant pressure waves to exceed 116 mmHg (Dinning et al., 2009b). This value represents the mean + 2SD of pressure wave amplitude in mid-colon in the *unprepared* bowel of healthy controls. If, however, we re-calculate the mean amplitude (+2SD) of propagating pressure wave at the level of the mid-colon in the prepared colon, we instead attain a value of 170 mmHg. If this were used to define HAPS in the prepared colon, the frequency of HAPS would be reduced to 14.6 ± 6.9 HAPS/24 h; this value does not differ significantly from HAPS frequency in the unprepared bowel. This is clearly highlights some of the inherent problems of comparing data recorded by different techniques.

One of the other major findings of this study is that bowel preparation influences pancolonic spatiotemporal patterns amongst consecutive PS over a 24 h period. Dinning *et al* previously proposed that continuity of flow of colonic content is dependent on the regional linkage between consecutive antegrade PS (Dinning et

al., 2009b). In this study, regional linkage was significantly reduced in the prepared colon, which supports the hypothesis that colonic content directly influences the organisation of pan-colonic PS. In the final 20-minute period immediately prior to defaecation, predefaecatory PS and HAPS frequency was equivalent between groups. However, the distal to proximal regional shift in the site of origin of PS leading up to defaecation (the stereotypical predefaecatory motor patterns), was mostly absent in the prepared bowel group.

There was also a significant increase in stool frequency in subjects with a prepared colon. Given that the volume and temperature of infused water were controlled in both groups, this is unlikely to be a reasonable explanation for this finding. In fact, all recorded episodes of defaecation during the study were only initiated after commencement of catheter perfusion (i.e. not during the 22 h recovery period immediately following intubation). This suggests that the distribution of perfused water into the colon differs significantly in a full, versus empty colon. In the cleansed bowel, the diffused fluid may collect more distally, resulting in a direct stimulatory effect which promotes defaecation. This assumption is in agreement with recent findings from a paediatric study, where radio-opaque markers used to determine CTT accumulated more distally in the prepared colon compared to an unprepared colon (Quitadamo et al., 2015).

It is also feasible that bowel preparation *per se* may sensitise the colonic mucosa and cause an augmented response to water perfusion. However, as the recording commenced approximately 40 h after bowel preparation was administered, it is unlikely that the intubation procedure itself and chemical agents had any impact upon the recorded data. Similarly, it is extremely unlikely that short acting drugs used during colonoscopy had any enduring effect.

This study is, however, not without limitations. One of the obvious criticisms is that both study groups were not sex- and age- matched in addition to possible differences in ethnicity and also in dietary and bowel habits between Australia and the UK. Nevertheless, it is unlikely that these factors could account for the noted manometric differences between study groups. In fact, previous epidemiological

studies have shown that stooling habits do not differ in the healthy elderly (Talley et al., 1992), and that there are no convincing effects of age alone on colonic transit, if the effects of other co-morbidity factors and inactivity are taken into consideration (Orr and Chen, 2002, Firth and Prather, 2002). Furthermore, meals were strictly standardised in both groups during the test period. Moreover, 80% of the volunteers recruited in Australia were found to be by chance backpackers from England or Europe and were therefore of similar ethnicity to the UK study group. To our knowledge, there are no data available in the literature to support the effect of any of the above factors on the specific PS characteristics that was the focus of this study. Furthermore, an increase in HAPS frequency, attributable to bowel preparation has previously been reported in a study using within subject comparisons (Lemann et al., 1995); it would seem unlikely that the same finding in the present study resulted from different subject cohorts rather than to removal of stool from the colon. Ideally, the current study would have been performed within one institution with a demographically-matched control group used. However, this was not possible, due to an inability to recruit those subjects who had previously undergone nasocolonic manometry in Australia.

In summary, colonic motor responses to commonly assessed physiological stimuli such as meals and morning waking are not influenced by bowel preparation. Therefore such responses can be compared in a valid way, among different studies within a laboratory or (within limits) among different laboratories. There are however, a number of quantitative and qualitative PS parameters (e.g. HAPS frequency and PS amplitude), as well as spatiotemporal organisation and regional linkage among consecutive PS that are influenced by bowel preparation. This must be taken into account when interpreting colonic manometric studies.

4 PANCOLONIC SPATIOTEMPORAL MAPPING REVEALS DISORGANISATION OF COLONIC PROPAGATING PRESSURE WAVES IN SLOW TRANSIT CONSTIPATION

4.1. INTRODUCTION

In STC, the principal pathophysiological mechanism is thought to be dysfunctional or deficient colonic propulsive motor patterns (Dinning et al., 2009a). As described previously (Chapter 1, section 1.3), motor activity of the colon, unlike that in the proximal gut, must cater for: prolonged storage in order to facilitate absorption of water and electrolytes; slow and stepwise transport of faecal content; and relatively infrequent evacuation of a substantial proportion of its total content. These discrete functions are likely to be associated with specific colonic motor patterns, which can be determined using colonic manometry. In health, manometric studies have identified propagating activity, defined as propagating sequences (PS) and have confirmed that these motor patterns are temporally linked to defaecation (Bampton et al., 2000) (Chapter 3), and intra-luminal transit of colonic content (Cook et al., 2000, Dinning et al., 2008a).

The characteristics of PS subserving distinct colonic functions appear important. PS associated with defaecation tends to be higher in amplitude and generally display a characteristic stereotypical relationship among consecutive PS (Bampton et al., 2000, Dinning et al., 2004) (Chapter 3). Nevertheless, low amplitude PS can at times move stool substantial distances along the colon (Cook et al., 2000, Dinning et al., 2008b). Other studies have demonstrated regional variation in PS activity along the colon (Cook et al., 2000, Bampton et al., 2001, Dinning et al., 2004) (Chapter 3).

In STC, relatively few manometric studies have been published since the first report three decades ago (Bassotti et al., 1988). The majority have only recorded motor activity from sites distal to the mid-transverse colon, and many are confined to the descending or sigmoid colon only (see chapter 1, Table 1.03). From these studies, alterations in PS frequency, and specifically decreased frequency of high amplitude PS (HAPS) has been implicated in the pathogenesis of STC (Bassotti et al., 1988, Di Lorenzo et al., 1992, Leroi et al., 2000, Hagger et al., 2003, Rao et al., 2004). However, an emphasis solely on indices of PS frequency and amplitude may be simplistic, given those the contemporary studies which suggest that spatiotemporal

organisation of PS activity may be as important if not more relevant to transit and stool expulsion (Dinning et al., 2009a) (studies of Chapter 3). The morphological characteristics, responses to physiological stimuli and spatiotemporal organisation among PS have never been defined throughout the entire colon of patients specifically with STC.

4.2. STUDY AIMS

This study aims to establish detailed spatiotemporal maps of PS activity from the caecum to the anorectum in STC, and to draw comparison with those observed in healthy controls.

Specifically, we hypothesised that: (**i**) derangements in motor patterns underpinning STC are multifactorial and that deficient, disrupted or disorganised spatiotemporal patterning among consecutive colonic PS exist in this condition; and (**ii**) pan-colonic, 24h spatiotemporal pressure mapping reveals one or more recognisable manometric 'signatures' that can serve as biomarkers of the disease.

4.3. MATERIALS AND METHODS

4.3.1. STUDY POPULATION

As severe STC in adults almost exclusively effects females (Knowles et al., 2003), this study included only female subjects.

4.3.1.1. Healthy volunteers

Colonoscopic- assisted water-perfused manometric catheter placement was carried out in eight healthy female volunteers in the UK (median age: 34 (range: 24 - 56) years) at the Royal London Hospital. These subjects had all previously been enrolled in the research study described in Chapter 3. Subjects were all recruited by advertisement and all fulfilled general inclusion criteria (Chapter 2, (2.3.1)).

4.3.1.2. STC patients

Colonoscopic- assisted water-perfused manometric catheter placement was carried out in in fourteen female patients (median age: 45 (range: 18 - 72) years) in Australia. For inclusion in the study, STC patients had to fulfil the general patients inclusion criteria (Chapter 2 (2.3.2)). Delayed colonic transit confirmed by isotope colonic transit study performed in the St George Hospital, Sydney, Australia (Chapter 2 (2.6.1.5)) (Smart et al., 1991, McLean et al., 1992). Inclusion and exclusion criteria for STC patients were reviewed and documented on site by the Australian group (Dr Phil Dinning).

All study subjects had given written informed consent to participate.

4.3.2. COLONIC MANOMETRIC TECHNIQUE AND INTUBATION

The water-perfused catheters and recording system have been described in detail previously [Chapter 2 (section 2.5.2)].

Healthy controls

On the day prior to intubation, healthy controls were allowed clear fluids only and bowel preparation was performed using oral administration of two Bisacodyl tablets and 250 mL magnesium citrate.

STC patients

On the day prior to colonoscopic insertion of the manometry catheter, patients were allowed clear fluids only and advised to take their usual laxatives. Colonic preparation followed as per institutional policy: this involved oral administration of 2L of a polyethylene glycol (Golytely; Braintree Laboratories, Braintree, MA, USA). STC patients were then instructed to stop all laxatives during the study period and only resume them after colonic extubation.

All study subjects followed a similar study protocol, as previously described [Chapter 2 (section 2.5.2.3)].

4.3.3. DATA ANALYSIS AND PRESENTATION

See Chapter 2 section 2.5.2.4.

4.4. STATISTICAL ANALYSIS

A Mann Whitney U-test was used to make inferences about potential differences regarding PS and HAPS characteristics (frequency, amplitude, velocity, site of origin, and extent of propagation) between STC patients and controls. The comparisons between these variables were made for the total colon, and for the proximal colon (ascending and transverse colon) and distal colon (descending and sigmoid colon). A non-parametric test for between-group comparisons was used because the normal distribution of the data was not always apparent. The same test was used to compare the regional linkage that existed between the two groups. Comparisons between the basal and the postprandial PS characteristics within subjects were performed using a paired *t*-test. Comparison between the delta values (basal - postprandial) between the patients and the control groups were performed using the Mann Whitney U-test. All data are expressed as mean ± SD. A *P* value of less than 0.05 was considered statistically significant.

4.5. RESULTS

5.5.1. SYMPTOM DURATION AND ISOTOPE RETENTION IN STC PATIENTS

The age, duration of symptoms, patient satisfaction with weekly bowel motions and isotope retention at 72 h are detailed in Table 4.01.

Patient No.	Age	Duration of symptoms (years)	Feeling of complete evacuation (days/ week)	Isotope retention% (72 h)
1	48	>10	1.2	100
2	58	>10	0	97.5
3	44	>10	0	89
4	18	2	1	80.2
5	58	>10	0.3	99.9
6	27	>10	1	66.7
7	48	>10	0	100
8	44	>10	0.7	100
9	48	>10	0.3	57.6
10	21	5 - 10	0	59.7
11	72	>10	0.7	95.2
12	29	2	0	100
13	31	5 - 10	1.3	100
14	45	>10	1.7	73.2

Table 4.01. Characteristics of slow transit constipation patients. Feeling of complete evacuation was obtained from the 3 weeks stool diary from patients. Isotope retention for colonic transit studies was calculated from the entire colon on day 3 (normal <9%). Only patients with >50% isotope retention were enrolled in the study.

4.5.2. PANCOLONIC MANOMETRY: 24 H SPATIOTEMPORAL ORGANISATION OF PROPAGATING SEQUENCES

In all STC patients and controls, colonoscopic assisted catheter placement was achieved without complication. Twenty-four hour manometric studies were completed in all subjects. Some subjects reported a transient lower abdominal "unease" upon catheter removal, but there were no other complications reported.

Twenty-four hour spatiotemporal maps revealed consistent and striking differences in frequency, distribution, extent and polarity of colonic propagating sequences in patients with STC compared with controls (Figures 4.01 and 4.02). Although the frequency of antegrade PS in the proximal and distal colon was similar between groups, there was a notable increase in the frequency of retrograde PS in STC patients. While substantial numbers of PS originated both in the proximal and distal colon of patients, these events only propagated over short distances and manifested as a loss of regional linkage among PS along the colon, with a virtual absence of propagating pressure waves within a relatively adynamic mid-colonic zone. The proportion of regionally linked antegrade PS in patients (40 ± 7%) was significantly lower when compared with controls (59 ± 9%; $P< 0.001$). The patient group also demonstrated a marked reduction in the amplitude of pressure waves throughout the colon. Additional features readily appreciated from spatiotemporal maps of the STC patients included lack of the normal nocturnal suppression of PS and the absence of the normal meal response.

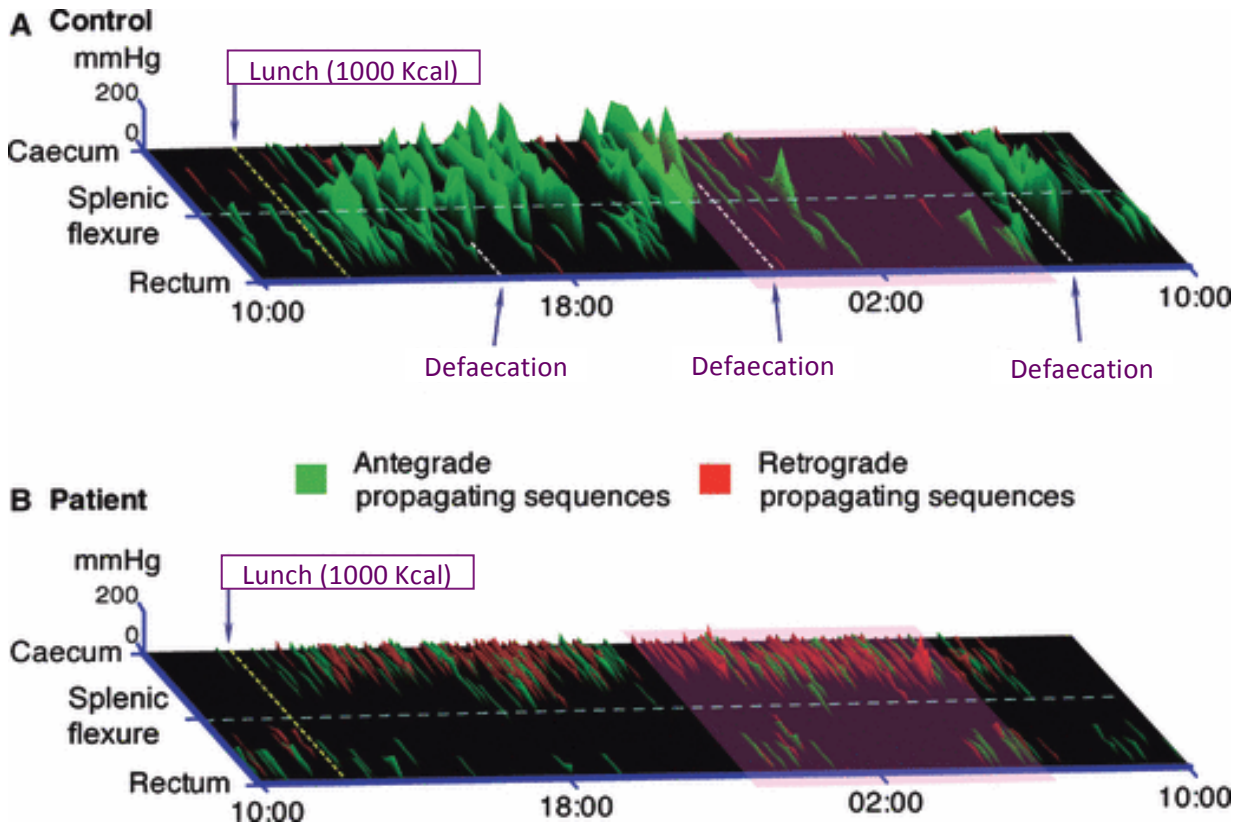


Figure 4.01. Twenty-four hour pan-colonic spatiotemporal maps of colonic propagating sequences (PS) in (A) a healthy control and (B) a female patient with STC. Within each map, each coloured ridge represents an antegrade (green) or retrograde (red) PS. The antegrade PS originate at the orad end of the green ridges, and retrograde PS originate at the anal end of the red ridges. The proximal margin of each antegrade and distal margin of each retrograde ridge indicates the precise site and time of origin of that PS. The axial length of the ridge indicates the extent of propagation. On each map the timing of the 1000 kcal lunch (yellow-hatched line) and defaecation (white-hatched line) is highlighted; and the horizontal blue-hatched line is the mid-colon (splenic flexure). The pink shading highlights the nocturnal period (22:00 - 06:00 h). The features immediately apparent from these spatiotemporal maps that distinguish patients from controls are: the marked paucity of PS in the mid- colon as a consequence of a significant decrease in the extent of propagation of antegrade PS originating in the proximal colon; a lack of pressure waves throughout the colon and the lack of the nocturnal suppression of PS and the normal meal response in the STC patient.

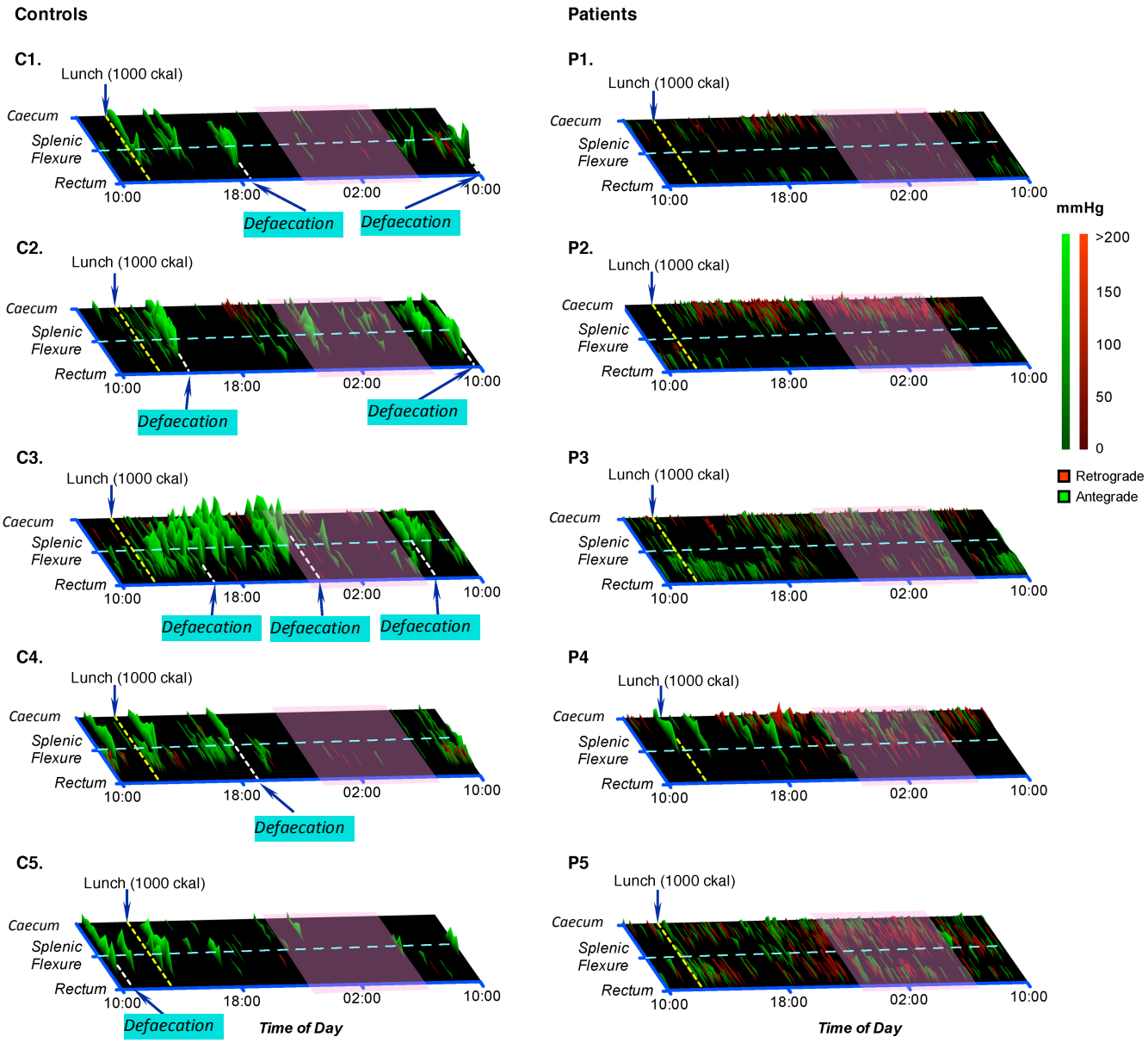


Figure 4.02. 24 hour spatiotemporal maps in five healthy controls (C) and another five patients (P) with slow transit constipation (STC). Similar abnormalities in PS characteristics that are detailed in Figure (4.01) are displayed in each of the patients with STC. Note- control C3 and patient P2 is the healthy subject and STC patient display in Figure 4.01.

4.5.3. DIURNAL VARIATION IN PROPAGATING SEQUENCES

All controls demonstrated normal nocturnal suppression of antegrade PS, with a mean decrease of 54 ± 26% from a daytime frequency of 5 ± 3 PS / h to a night time frequency of 2 ± 2 PS / h; (*P* = 0.01) (Figures 4.02 and Figure 4.03). This pattern was not displayed in STC patients (day: 6 ± 4 vs. night: 6 ± 4 PS h^{-1}; *P* = NS) (Figures 4.02 and 4.03).

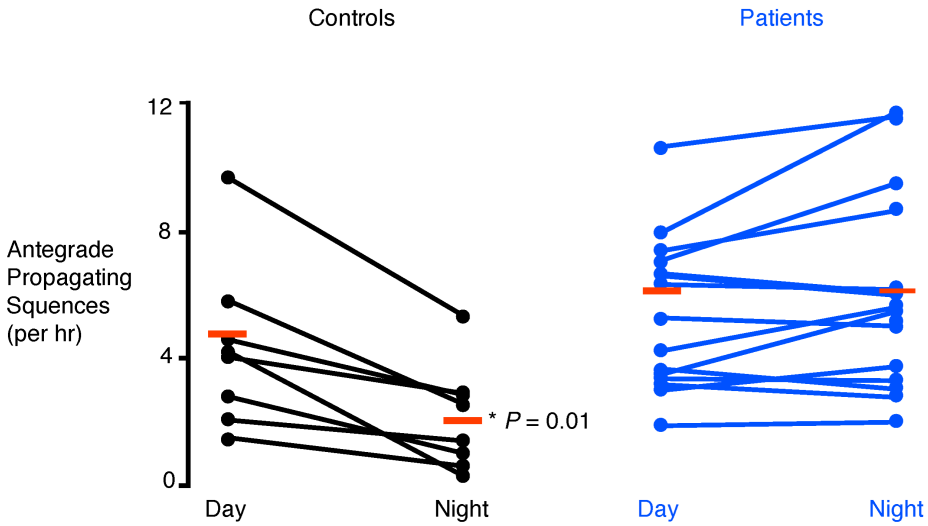


Figure 4.03. Frequency of antegrade propagating sequences. The number of antegrade PS per hour in each of the controls (n= 8) and STC patients (n= 14) during the day (06:00 - 22:00 h) and at night (22:00 - 06:00 h) are shown. In healthy controls, there was a significant decrease (*P*< 0.01) in propagating activity at night. This pattern was not observed in the majority of patients.

4.5.4. PROPAGATING SEQUENCE (PS) CHARACTERISTICS

The overall frequency of antegrade PS did not differ between groups (Table 4.02). When compared with controls, the amplitude of PS in STC patients was significantly lower ($P< 0.0001$) (Figure 4.04A; Table 4.02) and the extent of propagation of PS originating in the proximal colon was significantly reduced ($P = 0.0007$) (Figure 4.04A; Table 4.02). As noted above, the majority of PS originating in the ascending colon of patients did not extend beyond the distal transverse colon (Figures 4.01 and Figure 4.04A), resulting in a markedly reduced density of propagating pressure waves (PPW) in the mid-colon of STC patients (52 ± 24 PPW / 24 h) in comparison to controls (123 ± 52 PPW / 24 h; $P = 0.01$). When compared with controls, the overall frequency of retrograde PS was significantly higher in patients ($P = 0.03$) (Figures 4.01 and 4.02; Table 4.02). This was particularly evident in the proximal colon of patients, where a fourfold increase in retrograde PS was observed in comparison with control subjects (56 ± 67 vs. 13 ± 12/ 24 h in controls; $P = 0.04$) (Figures 4.01 and 4.02; Table 4.02). There was no significant difference in the amplitude, velocity or extent of propagation of retrograde PS between STC patients and controls (Table 4.02).

		Control (n= 8)			STC patients (n= 14)		
Colonic regions		Right colon	Left colon	Total colon	Right colon	Left colon	Total colon
Antegrade propagating sequences	Frequency/24 h	58±34*	38±30	96±60	72±44	62±43	135±72
	PPW/24 h	258±153	207±151	465±284	243±158	236±157	479±251
	Amplitude (mmHg)	62±14*‡	78±22‡	68±15‡	31±8†	32±6†	31±6†
	Extent of propagation (cm)	34±6*‡	21±2	29±5†	21±6	17±2	20±4
Retrograde propagating sequences	Frequency/24 h	13±12	15±17	27±24	56±67†	21±21	78±74
	PPW/24 h	59±53	65±61	101±95	197±240	48±29	246±246
	Amplitude (mmHg)	29±5	34±8	32±6	27±6	26±5	27±4
	Extent of propagation (cm)	18±3	20±3	19±3	19±3	20±4	18±2

Table 4.02. Antegrade and retrograde propagating sequence characteristics. For ease of presentation all significant differences are presented as $P< 0.05$. * A significant difference ($P< 0.05$) between the left and right colon within controls or patients. † A significant difference ($P< 0.05$) between control and patient groups for the same region. ‡ A significant difference ($P< 0.05$) between antegrade and retrograde characteristics within controls or patients for the same region.

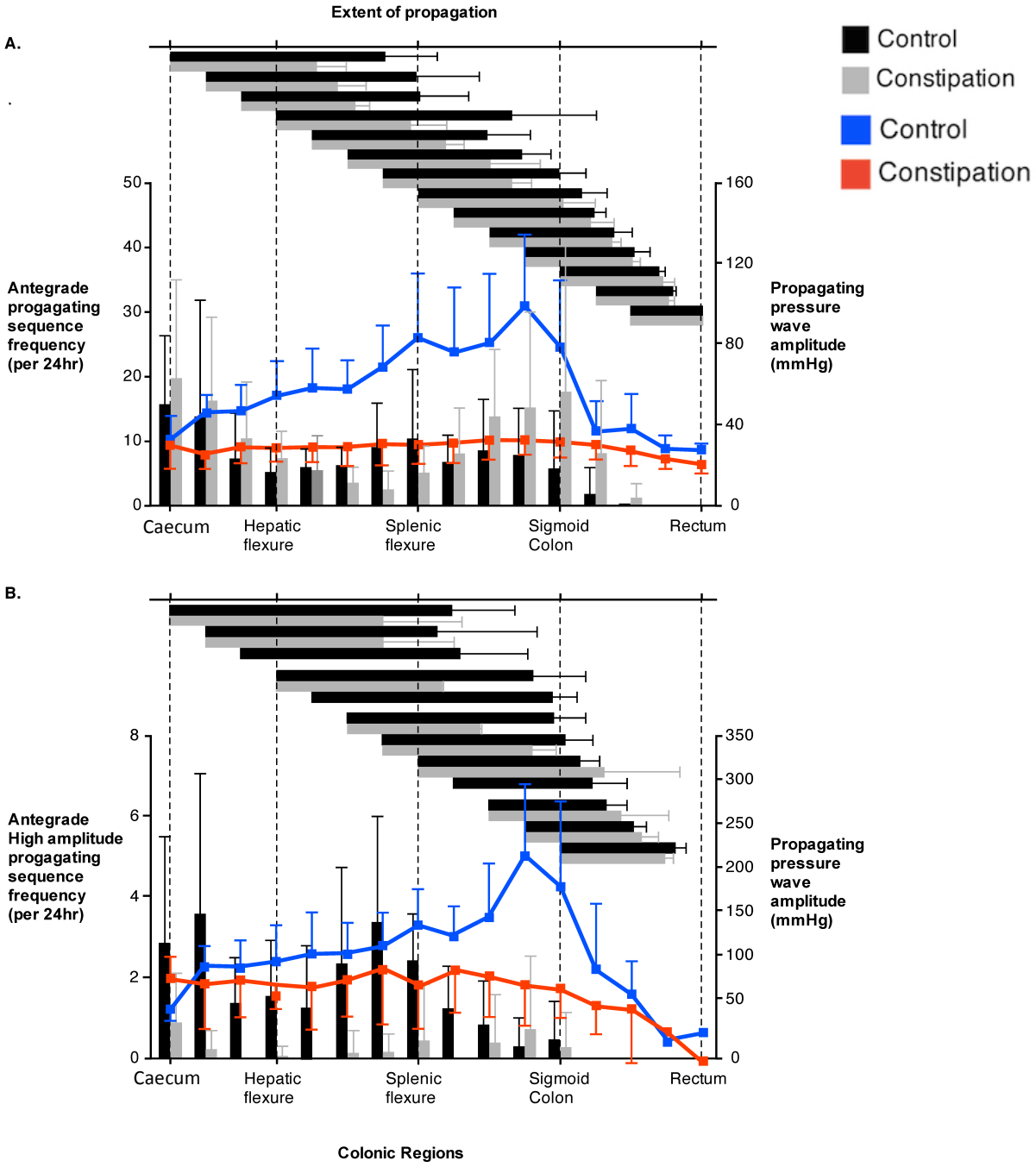


Figure 4.04. Regional variation in the frequency, amplitude and extent of propagation of antegrade propagating sequences (PS) panel (A), and of high amplitude PS (panel B) in controls (n= 8) and patients (n= 14). The vertical bars show the frequency distribution of PS grouped according to the site of origin. The horizontal bars show the mean extent of propagation according to the site of origin. The solid blue (control) and red (patient) lines indicate the mean amplitude of the component pressure waves at each colonic region. (**A**) When compared with controls, note the significant reduction in pressure wave amplitude ($P< 0.0001$) and the extent of propagation of PS throughout the colon in patients ($P = 0.001$). (**B**) High amplitude PS frequency is markedly reduced in patients ($P< 0.0001$). High amplitude PS generated in the proximal colon of patients, extend significantly shorter distances along the colon when compared with controls ($P< 0.0001$).

4.5.5. HIGH AMPLITUDE PROPAGATING SEQUENCES

Antegrade HAPS were recorded in all controls but in only 10 (71%) patients (Figures 4.01 and 4.02). In controls, HAPS were initiated five times more frequently in the proximal colon than in the distal colon ($P< 0.0001$) (Figures 4.01 and 4.04B). This regional difference was not seen in patients. Overall, patients exhibited a significant reduction in the frequency ($P< 0.0001$), amplitude ($P< 0.0001$), and extent of propagation of HAPS ($P< 0.0001$) (Figures 4.01 and 4.04B) when compared with controls (Table 4.03).

	Controls			STC patients		
Colonic regions	**Right colon**	**Left colon**	**Total**	**Right colon**	**Left colon**	**Total**
Frequency/24 h	17±7*	3±3	21±8.2	2±3†	2±4	4±6†
Amplitude (mmHg)	104±22	147±29	125±19	75±32†	68±12†	78±21†
Extent of propagation (cm)	43±6*	21±4	38±5	36±15†	20±16	3±16

Table 4.03. High amplitude propagating sequence characteristics. For ease of presentation all significant differences are presented as $P< 0.05$. * A significant difference ($P< 0.05$) between the left and right colon within controls or patients. † A significant difference ($P< 0.05$) between control and patient groups for the same region. ‡ Note that a PS was classified as a HAPS if the amplitude of at least one component propagating pressure wave was > 116 mmHg. Many pressure waves in the sequence do not exceed 116 mmHg and this reduces the average amplitude.

4.5.6. COLONIC MEAL RESPONSE

A postprandial increase in HAPS frequency was recorded in seven of eight healthy controls but in only 2 of 14 (14%) STC patients ($P = 0.003$) in response to a standard 1000 kcal meal (Figures 4.01 and 4.02). One healthy control defecated immediately prior to lunch and this event was preceded by HAPS. In this subject, HAPS frequency was the same prior to and after the meal. The meal-related increase in

HAPS frequency seen in controls (3.3 ± 2.8 HAPS/2 h) was significantly blunted in the patient group (0.1 ± 0.7 HAPS/2 h; *P* = 0.01). The increase in HAPS frequency observed was not specific to any particular 30 or 60 minutes postprandial epoch.

4.5.7. DEFAECATION

No STC patient defecated during the recording period, whereas all controls did (*P* 0.0001), mostly passing moderate amounts of watery stool (as previously described in Chapter 3). Timing of defaecation in one healthy volunteer was unknown based on diary entries, and this subject was removed from this analysis only. Twenty one episodes of defaecation were recorded in the remaining seven healthy controls, and stool expulsion was deemed to be directly associated with 54 (35%) of the 154 HAPS recorded in these subjects (Figures 4.01 and 4.02).

4.6. DISCUSSION

Measurement of *in vivo* colonic motor function is fundamental to provide a better understanding of the pathophysiological mechanisms underlying chronic constipation (including STC), However, direct assessment (as opposed to indirect measurement through colonic transit studies) of human colonic motility poses substantial methodological challenges (see Chapter 1, section 1.6.1.3.1.2). Therefore, our understanding of the physiology of motor patterns and pathophysiology of subtypes of chronic constipation remains relatively primitive compared to other parts of the GI tract. Previous colonic manometric studies have attempted to describe colonic motor dysfunction in chronic constipation (Bassotti et al., 1988, O'Brien et al., 1996, Ravi et al., 2010). However, none of these studies provided information derived from the proximal colon.

To our knowledge, the current study has demonstrated for the first time a number of potentially important findings in patients with STC that were not observed in healthy volunteers. This had been achieved using the pan-colonic monomeric assessment with spatiotemporal maps used to visually display the spatiotemporal distribution of PS throughout the entire colon over a 24 h period.

The novel observations in STC were as follows: (1) presence of a relatively adynamic region around the splenic flexure, secondary to a visually evident loss of regional linkage, along with marked decrease in the extent of propagation of antegrade PS in the proximal colonic regions; (2) an increased frequency of proximal colonic retrograde PS; (3) absence of the normal nocturnal suppression of antegrade PS that was observed in healthy subjects. In agreement with previous studies, our study also confirmed an absent meal response in most STC patients (Hagger et al., 2003, De Schryver et al., 2003, Rao et al., 2004, Herve et al., 2004). Further, one third of the STC patients in this study displayed a reduction in PS frequency, along with a reduction in the extent and the amplitude of HAPS (Bassotti et al., 1988, Leroi et al., 2000, Hagger et al., 2003).

Studies led by Dinning in Australia, as well as those described in Chapter 3, have shown that in healthy subjects there is regional variation in the distribution and frequency of PS between the proximal and the distal colon and also co-ordination of PS activity across these regions (Bampton et al., 2001, Dinning et al., 2004, Dinning et al., 2009b). Accordingly, pan-colonic manometric assessment is essential to determine potential differences throughout the entire large bowel between healthy subjects and patients who suffer from STC.

Patients with STC are defined by a delay in transit through the colon (and perhaps other regions of the GI tract (Zarate et al., 2009). Contrary to previous studies which almost exclusively proposed reduced HAPS frequency as the principal underlying pathophysiology (Chapter 1, section 1.6.1.3.1.8), this study showed PS frequency overall was similar between controls and STC patients. It may be that delayed transit in some STC patients can be explained by the observed marked disturbances in distribution, polarity (more retrograde PS) and organisation of PS, leading to *dysregulated*, rather than a reduction in overall colonic motor function. An increase in proximal colonic retrograde PS activity has previously been shown in patients presented with significant symptoms of obstructed defaecation (Dinning et al., 2004). Retrograde PS activities are able to propel colonic content to more proximal colonic regions (Dinning et al., 2008a), which ultimately likely causes delayed emptying of proximal colonic content. The resultant increase in residence time potentially leads to further fluid absorption and a firmer colonic content that would be more difficult to propel distally than softer content. The observed short extent of propagation of antegrade proximal colonic PS and the overall reduced linkage between proximal and distal colonic PS both further explain delayed transit in STC patients. One alternative mechanism is the presence of distal colonic ‘obstruction’, secondary to the presence of hard stool in the distal colon, which could induce a reduction in proximal colonic motility through feedback inhibition (Bampton et al., 2002), as observed previously in the unprepared colon. However, this is unlikely to be the case with regard to these study findings, as prior bowel preparation had cleared all solid colonic content as confirmed during colonoscopic - assisted catheter placement. Furthermore, all enrolled STC patients had evacuation proctographic examination

that excluded the presence of significant functional and anatomical rectal obstructive features.

The underlying cause of colonic dysmotility displayed in these patients is unknown. However, central neuronal dysregulation may be one proposed mechanism. This study showed a relative absence of the normal nocturnal inhibition of antegrade PS. The regulation of diurnal variation in colonic motor activities (nocturnal suppression followed by waking related stimulation of PS) has previously been linked to central nervous system control (Furukawa et al., 1994). The observed attenuation of the sleep response may indicate a central neuropathic cause (Rao et al., 2004).

Previous histological studies have reported a reduction in the density of interstitial cells of Cajal (Lyford et al., 2002) the 'pacemaker' of the gut, as well as a decrease in the overall population of glial cells (Bassotti et al., 2007) in constipated patients. These findings may support those of the current study in terms of the spatiotemporal disorganisation among consecutive PS. Another interesting finding was he quiescence of the PS activities around the splenic flexure which represents the embryological junction between the midgut and the hindgut (Sadler et al., 1985), and these two embryonically distinct regions have different blood and neural supplies and have been shown to differ in the expression of genes and antigens (Bufill, 1990, Glebov et al., 2003). In patients presenting with STC, this junction may potentially represent a site of disrupted intrinsic neural supply that may express as a loss in organised motor activities. Most of the STC patients enrolled in this study reported long history of constipation (> 10 years) and this proposed mechanism may most relevant to those patients with near lifelong symptoms. Whether similar colonic dysmotility is present in patients who develop their symptoms of STC later in life and / or after pelvic surgery or childbirth (Knowles and Martin, 2000) remains unknown. A recent paediatric study using a water-perfused colonic manometry technique, attempted to correlate manometric findings with histological results obtained from segmental colonic resection in 18 children suffering from refractory severe STC (Giorgio et al., 2013). Neuropathic abnormalities reported to be the predominant histopathological findings in paediatric STC patients. The study also showed that

following administration of intraluminal bisacodyl, the majority of STC patients display reduced frequency of HAPS, increase in the LAPS, increase in the non propagating activities, and no difference in the motility index from the control group (Giorgio et al., 2013). These findings are in agreement with our study results that showed dysregulated motor activities rather than an overall reduction in colonic motor activities.

This study has also shown some contrasting results to previous studies (Hagger et al., 2003, Rao et al., 2004), The discrepancy in nocturnal findings between our study and earlier studies is likely to reflect a differences in recording technique. For example, Rao *et al* demonstrated the presence of normal nocturnal inhibition of colonic motor patterns (Rao et al., 2004); however, that study reported no data from the proximal colon. The study also used a generalised 'area under the curve' measurement to define motility at night, and did not measure individual PS. In the study by Hagger *et al* (Hagger et al., 2003), data were recorded from the ascending colon, however, their catheter had only five sensors spaced at 15 cm and such spacing would miss the majority of all propagating activity recorded here. Perhaps because of this, a 'motility index' was used to define nocturnal activity. Hence, neither study is directly comparable.

The ability of any test of colonic function, including colonic manometry, to help identify specific biomarkers of disease that can differentiate sub-types of constipation and ultimately guide and improve treatment and predict outcomes in these patients, still remains elusive. The American Neurogastroenterology and Motility Society consensus paper (Camilleri et al., 2008) stated that "there are no published quantitative data of phasic contractility, that unequivocally differentiate normal colonic function from colonic inertia". Although the current study reports highly significant quantitative differences between patients and controls, that consensus statement remains true, as some of these findings may be found in other subgroup of patients (Dinning et al., 2004, Dinning et al., 2009b). However, the possibility that subsets of patients may demonstrate specific colonic manometric findings that might be predictive of therapeutic outcome remains a target for future studies.

There are a number of potential criticisms with this study. These include age differences, and the possibility of geographic confounders (including feeding habit and ethnicity) being introduced, as the studies were carried out in two geographically different sites. However, these issues have previously been addressed in detail in Chapter 3 (section 3.6). Further, type of bowel preparation used in both groups was different, as each centre followed bowel preparation protocol as per recommended by the hospital. Given that similar study protocol and equipment were standardised between the groups, it is improbable that any differences recorded between the groups were due to differences in bowel preparation.

In summary, this study has demonstrated the utility of spatiotemporal mapping to condense and display in a readily interpretable format, a number of new and potentially important disturbances in the spatiotemporal organisation of PS in patients with STC. Given the importance of PS to normal transit and the process of defaecation, these abnormalities are pathophysiologically relevant. Whether these markers of dysmotility, or combinations of them, might represent true disease 'biomarkers' will require further systematic evaluation in a wider range of studies.

5 MANOMETRIC ASSESSMENT OF PANCOLONIC MOTOR FUNCTION: COMPARISON BETWEEN SOLID-STATE AND WATER-PERFUSED TECHNOLOGIES

5.1. INTRODUCTION

There are currently two main types of catheter available for recording gastrointestinal pressure changes: water-perfused and 'solid-state'. Both systems have advantages and disadvantages (see Chapter 1, section **1.6.1.3.1.3**). However, the current standard for evaluating pancolonic motor function is water-perfused technology, which allows for easier and more cost-effective recordings. However, there are well-recognised limitations such as attenuated frequency response (Smout, 2001, Scott, 2003), the need for continuous equipment observation and maintenance, and that recordings are prone to artifacts, due to movement of the connecting tubing or air bubbles that can easily be created within the system as a result of fluid perfusion. With technological development and the introduction of microtransducer sensors that can be incorporated into the catheter assembly, a 'solid-state' pressure recording system provides an alternative method for recording intraluminal pressure changes. Such technology offers a better response to high frequency contractions (Smout, 2001, Scott, 2003), and enables ambulatory studies (as there is no need for continuous fluid perfusion), thus providing a more 'physiological' study environment. With regards to feasibility and practicality of using different catheter systems, a clinical trial compared the use of solid-state and water-perfused catheters in recording pressure activities within the sphincter of Oddi during endoscopic retrograde cholongiopancreatography (ERCP), and showed that the solid-state system was easier to use and set-up, provided more mobility to subjects, and carried less risk of infection during the procedure when compare to a water-perfusion recording system (Draganov et al., 2009). Furthermore, 24 h oesophageal ambulatory solid-state studies have shown a better diagnostic yield of abnormal oesophageal body motility compared with stationary water-perfused studies (Chrysos et al., 2002). More recently, solid-state catheters incorporating a higher number of pressure sensors (high resolution manometry) have become available for clinical use in assessing upper GI function, allowing better understanding, technique standardisation, and classification of oesophageal motility disorders (Chicago classification) (Bredenoord et al., 2012).

In lower gut studies, the use of solid-state catheters for measuring colonic pressure activities has generally been limited to the distal colon (rectosigmoid region) (Kamm et al., 1992b, Ferrara et al., 1994, Rao et al., 2001b, Hagger et al., 2003, Rao et al., 2004). Only a few studies have recorded colonic contractile activities from more proximal colonic regions using this type of catheter, but with a very limited number of pressure sensors (Chapter 1, Table 1.03). Such catheter configurations would undoubtedly fail to detect a significant proportion of pressure contractile activities, due to long inter-sensor distances (Scott, 2003). Soffer *et al* for example, was the first to attempt to record pancolonic motility using a solid-state catheter incorporating only 3 pressure sensors (Soffer et al., 1989). Nevertheless, increasing the number of sensors with closer spacing, akin to the catheter configuration used for water-perfused manometric assessment of pancolonic pressure activities (see Chapter 3) offers the opportunity to record pancolonic motor function in conditions that better represent the organ's physiological status (i.e. no confounders resulting from water perfusion or non-ambulation status). The effect of bowel preparation has been addressed previously in chapter 3. To date, however, there is no reported study that has compared recordings obtained from the whole colon using both manometric techniques in adults. In one animal study, in which both solid-state and water-perfused catheters were inserted into a canine colon, it was shown that recorded motility patterns were influenced by the recording techniques (Cook et al., 1988). For example, although both catheters were able to detect the majority of colonic contractions compared to a reference device (extraluminal serosal strain gauges), a significant number of colonic contractions (both phasic and tonic activities) were misrepresented, which was attributed to both asymmetry and wide diameter of the canine colon (Cook et al., 1988). In children, Liem *et al* have shown that recording of both HAPS and low amplitude pressure waves was influenced by catheter type (Liem et al., 2012).

Accordingly, in adult subjects, we aimed to evaluate the effects of varying recording technology on colonic motor activities by quantitative and qualitative analysis of recordings obtained from water-perfused and solid-state catheters within the same subjects and following a similar study protocol.

5.2. MATERIALS AND METHODS

5.2.1. STUDY POPULATION

Eight healthy subjects (all female), who had previously undergone a water-perfused manometry study in the prepared colon (Chapter 3), were invited to undergo a further solid-state manometry study. Additional eight healthy volunteers (all female) were also recruited by advertisement. The advertisement was placed on the Queen Mary College University of London website and circulated amongst college webmail users. Respondents attended a screening visit and direct interview. They all were asked to fill out a set of screening questionnaires prior to enrolment (see Appendices 2 - 4). During the screening visit, appropriate consent was obtained from all study subjects. Inclusion and exclusion criteria were applied as previously described (Chapter 2, section 2.3.1).

5.2.2. COLONIC MANOMETRIC TECHNIQUE AND EQUIPMENT

The recording of pancolonic motor function was performed using both a solid-state catheter (UniTip: Unisensor AG, Attikon, Switzerland) (Figure 5.01 and 2.02) and a water-perfused catheter (Dentsleeve, Wayville, SA, Australia) on separate occasions (Figure 2.05). Catheters were introduced into a prepared colon with the aid of a colonoscope, and advanced to the caecum as described previously [see Chapter 2, section 2.5.2.2) and Figures 2.08 and 2.09]. Catheter order was randomised apart from in those subjects (n = 8) who had previously undergone a water-perfused study. Study protocol was the same as described previously [Chapter 2, section 2.5.2.3].

For solid-state catheter studies, pressures were processed through 5 external connectors (each containing 4 pressure channels per connector) (UniTip: Unisensor AG, Attikon, Switzerland), with recorded signals being amplified and digitised at 6 Hz by a portable recorder (Flexilog, Oakfield Instruments Ltd, Eynsham, UK) (Figure 5.01). For water-perfused studies, this was achieved in a similar fashion as previously described using a customised MMS manometry system (Solar

Measurement System, software version 8.7b; Medical Measurement Systems, Enschede, the Netherlands) (Figure 5.02).

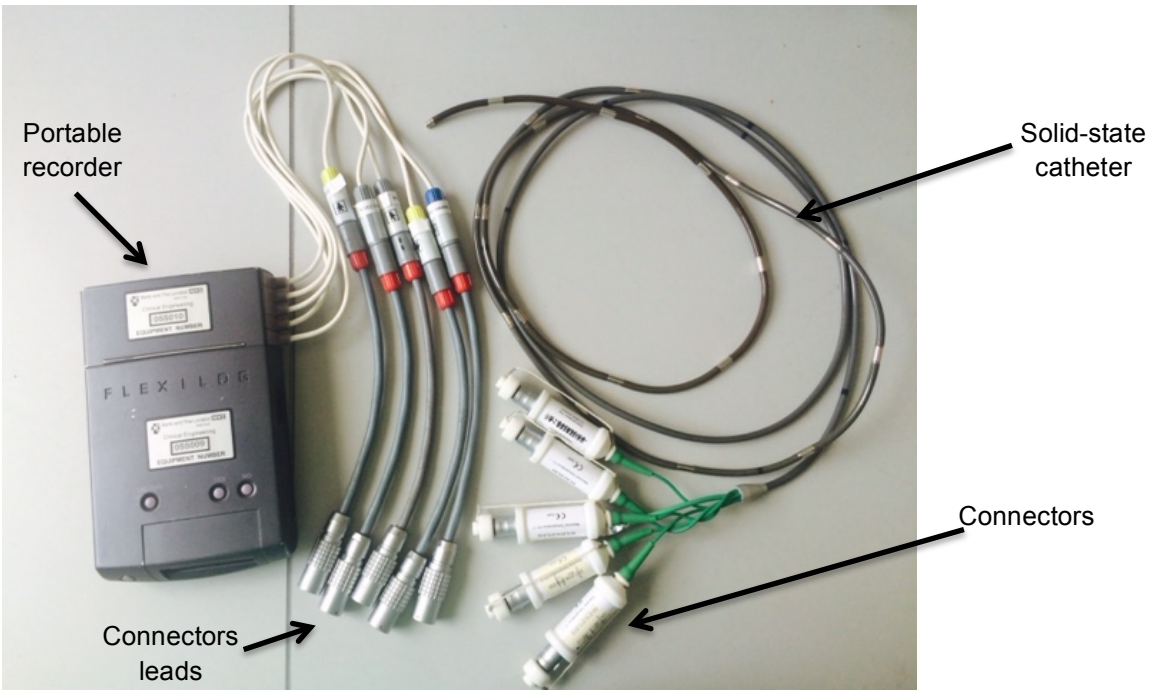


Figure 5.01. The solid-state catheter recording system. The portable recorder was carried in a shoulder harness for the duration of the recording.

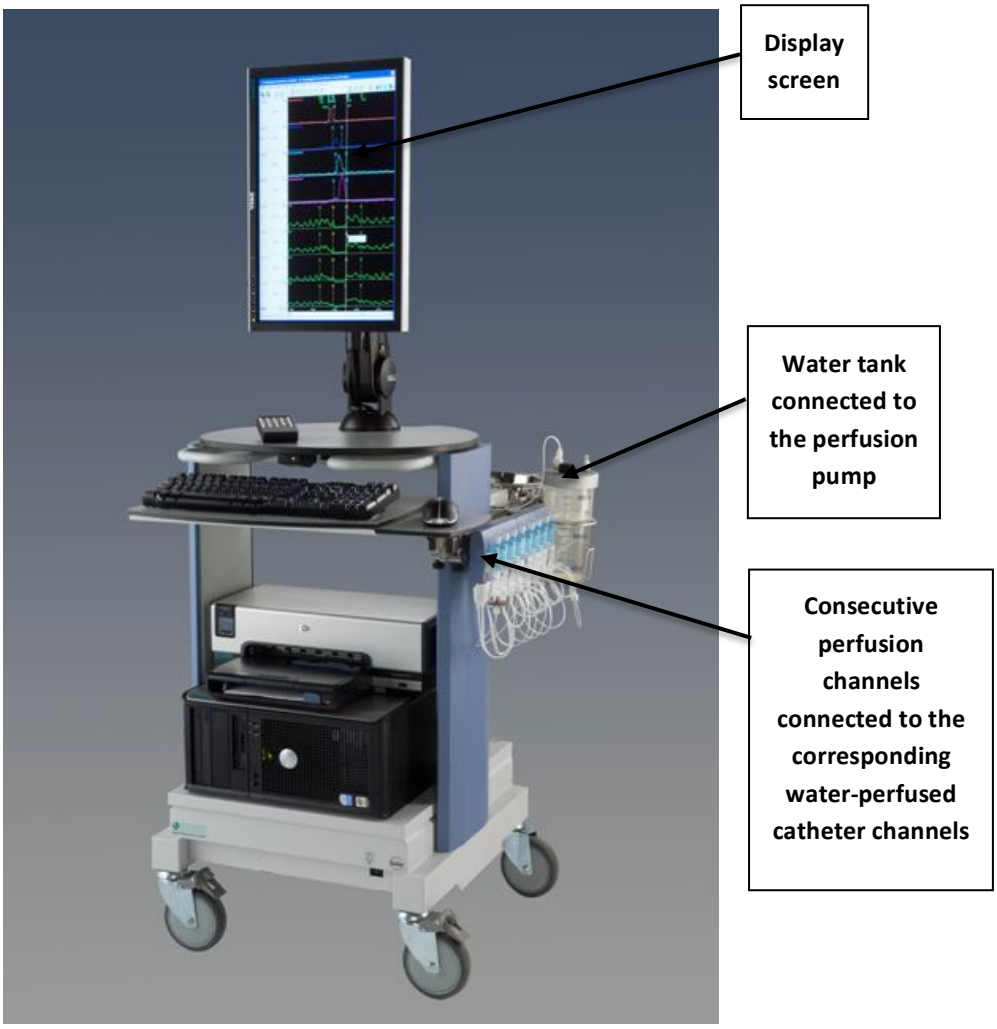


Figure 5.02. The water-perfused recording system (Solar Measurement System, software version 8.7b; Medical Measurement Systems, Enschede, the Netherlands). Subjects needed to be connected to the system for the duration of the study (24 h).

5.2.3. BENCH TESTING

In order to test the validity of comparing recordings yielded by different catheters, a bench test was performed to compare the 'rise-time' to an imposed pressure, and the maximum pressure amplitude recorded from both catheters under controlled conditions. A custom-built, airtight cylinder of sufficient length to introduce both catheters was utilised (UniTip: Unisensor AG, Attikon, Switzerland), to which

pressure was applied using a manual manometer. An impulse rise in pressure was applied to each catheter, starting with 25 mmHg, and then 50, 100, and 150 mmHg. The time from detecting pressure change to the peak of the recorded pressure was measured, as was the magnitude of peak pressure. Each catheter was linked to its appropriate recording and display system.

5.2.4. Data analysis

Qualitative analysis was performed based on visual description of observed motor activities. Quantitative analysis was performed as previously described [Chapter 2, section 2.5.2.4].

5.5. STATISTICAL ANALYSIS

As water-perfused and solid-state studies were performed within the same subjects, a Wilcoxon non-parametric paired *t* test was used to examine direct comparisons between all PS and HAPS characteristics (frequency, amplitude, site of origin, and extent of propagation) between groups. Data are expressed as median (interquartile range). The comparisons between these variables were made for the total colon, the right colon (ascending and transverse colon), and the left colon (descending and sigmoid colon). Comparison between basal and postprandial PS characteristics within subjects was performed with a paired *t* test; data are expressed as mean ± SD. A paired *t* test was also used to compare results of bench test studies. The Fisher's exact test was used to compare the number of episodes of stool expulsion associated with the stereotypic predefaecatory pattern of PS in each group. All statistical analyses were performed using GraphPad Prism (GraphPad software Inc., USA, version 5). A P value < 0.05 was considered statistically significant.

5.6. RESULTS

5.6.1. STUDY COHORT

Sixteen healthy subjects were recruited to the study. Ultimately, however, only 6 subjects (all female, median age 38 [25 - 56]) completed both manometric studies. Of the other 10 volunteers invited to take part, five declined testing after consent was obtained and the remaining five had to be excluded for methodological reasons: (i) in 2 subjects, there was major antegrade 'slippage' of the solid-state catheter during the second day of the study. This was deemed due to the weight of the external connectors, and was corrected for in subsequent recordings by better fixation; (ii), in 2 volunteers, colonic intubation of the solid-state catheter failed due to subject intolerance allied to sigmoid narrowing, as reported by the specialist performing the colonoscopy; (iii) in 1 subject, the solid-state catheter recorder could not be uploaded to the analysis software, due to an unexpected recorder error, secondary to failure in registering catheter calibration.

Studies were performed a median of 18 months apart (range 3 - 24 months).

5.6.2. CATHETER PLACEMENT AND STUDY CONDUCT

Overall, colonoscopically assisted intubation of the solid-state catheter was more time-consuming and more challenging in terms of reaching the caecum compared to the intubation using the water-perfused catheter. This was mainly due to catheter stiffness, which made it more difficult to pass around the splenic and hepatic flexures. Furthermore, the solid-state catheter was more prone to sensor damage as a result of difficult intubation and had to be sent for repair several times during the course of the study period. Nevertheless, the solid-state catheter was well tolerated by all subjects once in its desired position. Subjects were then able to ambulate freely, although movement was restricted as per water-perfused studies for the purpose of data analysis. No difficulties were encountered during water-perfused catheter intubation, and was well tolerated by all subjects.

Following intubation, all subjects who underwent solid-state studies reported that the portable recorder was easy to carry, compared to them being continuously connected to the water perfusion system; however, the external connecters were heavy and caused some minor discomfort, specifically during sleep. For water-perfused studies, generation of air bubbles within the catheter was observed mainly at the start of each study.

5.6.3. BENCH TESTING

The solid-state catheter recording system responded faster to all applied pressures compared to the water-perfused catheter assembly (25 mmHg: 3 ± 0.7 vs. 7 ± 2 sec, P = 0.03; 50 mmHg: 4 ± 1.1 vs. 8 ± 2 sec, P = 0.03; 100 mmHg: 4.4 ± 0.8 vs. 10 ± 3 sec, P = 0.03; 150 mmHg: 6 ± 2 vs. 12 ± 2 sec, P = 0.09) respectively. Maximum amplitudes recorded by the solid-state catheter relative to the applied pressure were slightly higher than those recorded by water-perfused catheter in spite of prior accurate calibration (25 mmHg: 23 ± 2 vs. 20 ± 2 mmHg; P = 0.2; 50 mmHg: 47 ± 3 vs. 40 ± 3 mmHg; P = 0.03; 100 mmHg: 92 ± 3 vs. 85 ± 6 mmHg; P = 0.07; 150 mmHg: 141 ± 11 vs. 128 ± 11 mmHg; P = 0.09). However, the solid-state catheter was only able to record pressure amplitudes to a maximum of 330 mmHg before experiencing a sudden drop in magnitude, followed by recovery once the applied pressure was below this level. Therefore, for the purposes of data analysis of *in vivo* studies, these attenuated drops in pressure were compensated for by identifying such drops manually and interpolating between the onset and offset of the drop by populating the data sheet with the last recorded pressure prior to the drop. This created a ‘flat top’ in some HAPS as shown in Figure 5.03.

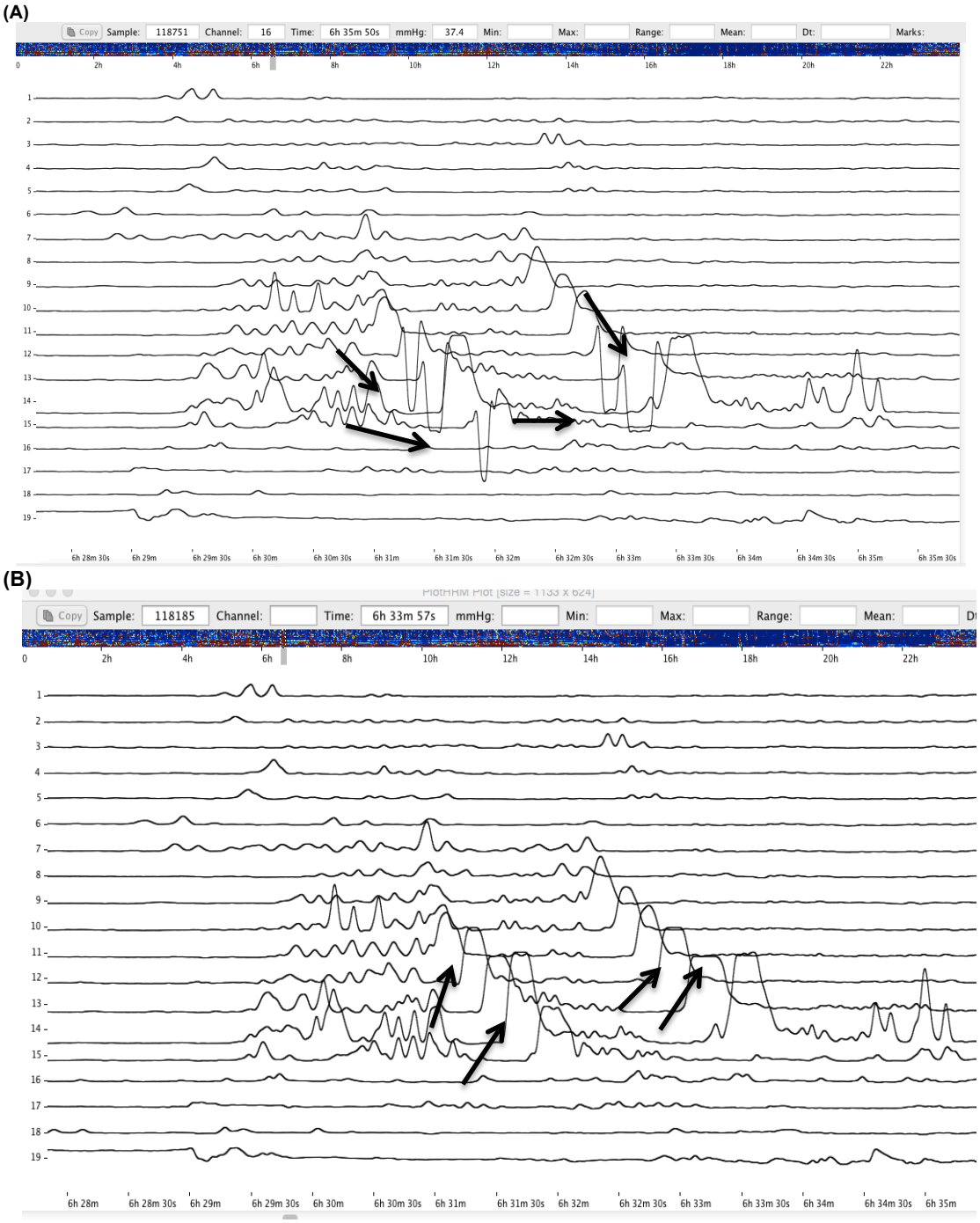


Figure 5.03. Correction of artefact allied to high amplitude propagating sequences (HAPS). (A) artificial pressure drop during a solid-state study (black arrows) highlighting a fall in pressure within the recorded HAPS from recording channels 12 to 15. This is secondary to a recording ceiling at pressure amplitudes greater than 330 mmHg; **(B)** shows the same HAPS after data interpolation. The x-axis represents the time line, while the Y-axis represent the channel position; the top channel is located within the caecal area, while distal channel represents the distal colon (rectosigmoid region). Seven minutes of recording are shown.

5.6.4. QUALITATIVE ASSESSMENT

In comparison to water-perfused studies, the colon showed near continuous motor activities over the entire recording period using solid-state technology. Qualitatively, pressure waves were observed more frequently in solid-state than water-perfused acquired studies (Figure 5.04), though it was not possible to accurately identify the polarity or propagation of many as shown in Figure 5.05. There was also a clear loss of suppression of colonic motor activities (including identified PS) throughout the entire colon during the nocturnal period when compared to water-perfused studies. This can be best appreciated by spatiotemporal colour-countered maps (Figure 5.06). Furthermore, retrograde PS activities were more evident in solid-state studies (Figure 5.06).

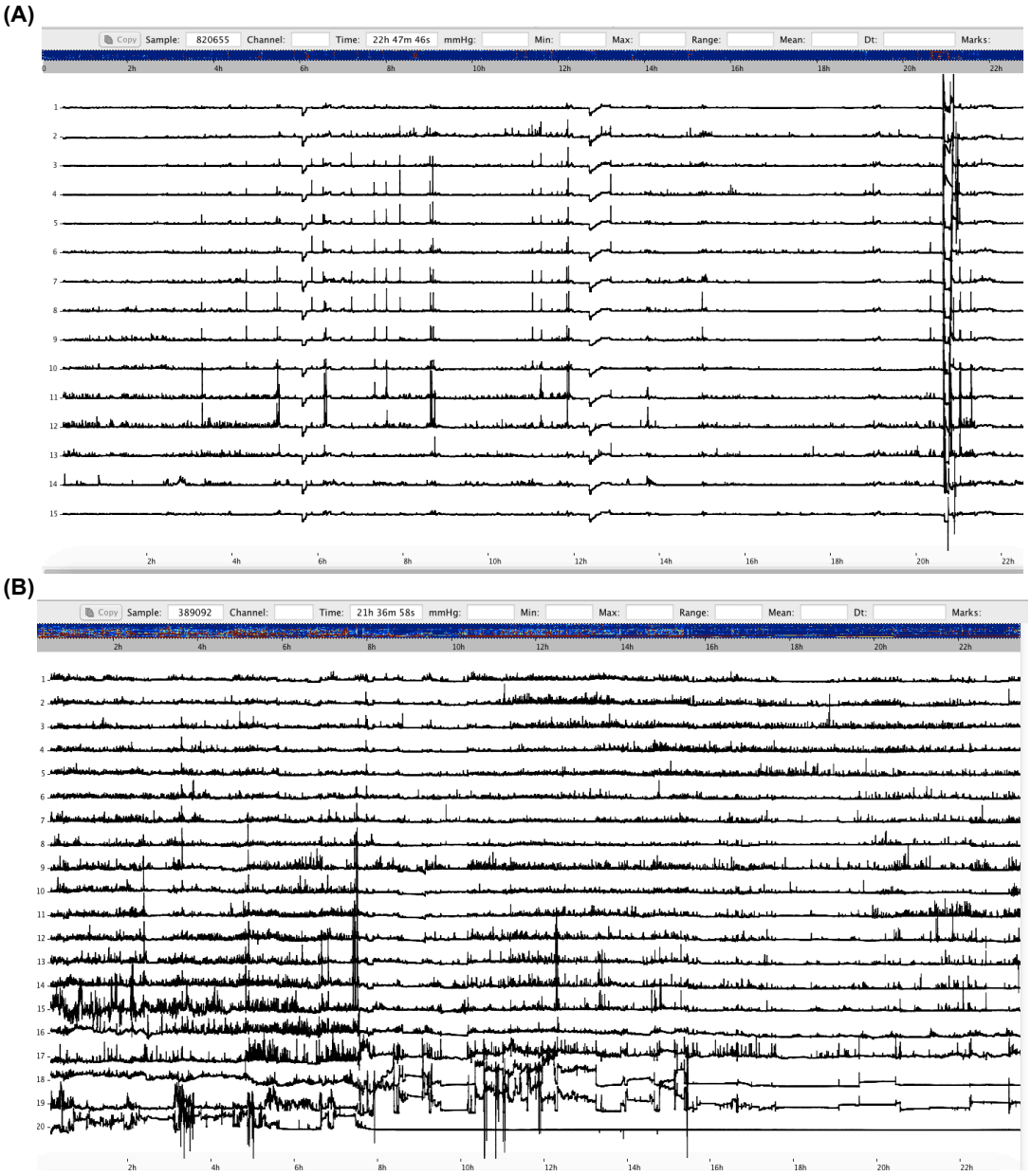


Figure 5.04. Examples of compressed overall colonic motor activities recorded over a 24 h period using (A) a water-perfused and (B) solid-state catheter systems. This highlights the increased levels of activity recorded with the solid-state catheter. The x-axis represents the time line, while the Y-axis represents the channel position; the top channel is located within the caecal area, while distal channels represents the distal colon (rectosigmoid region).

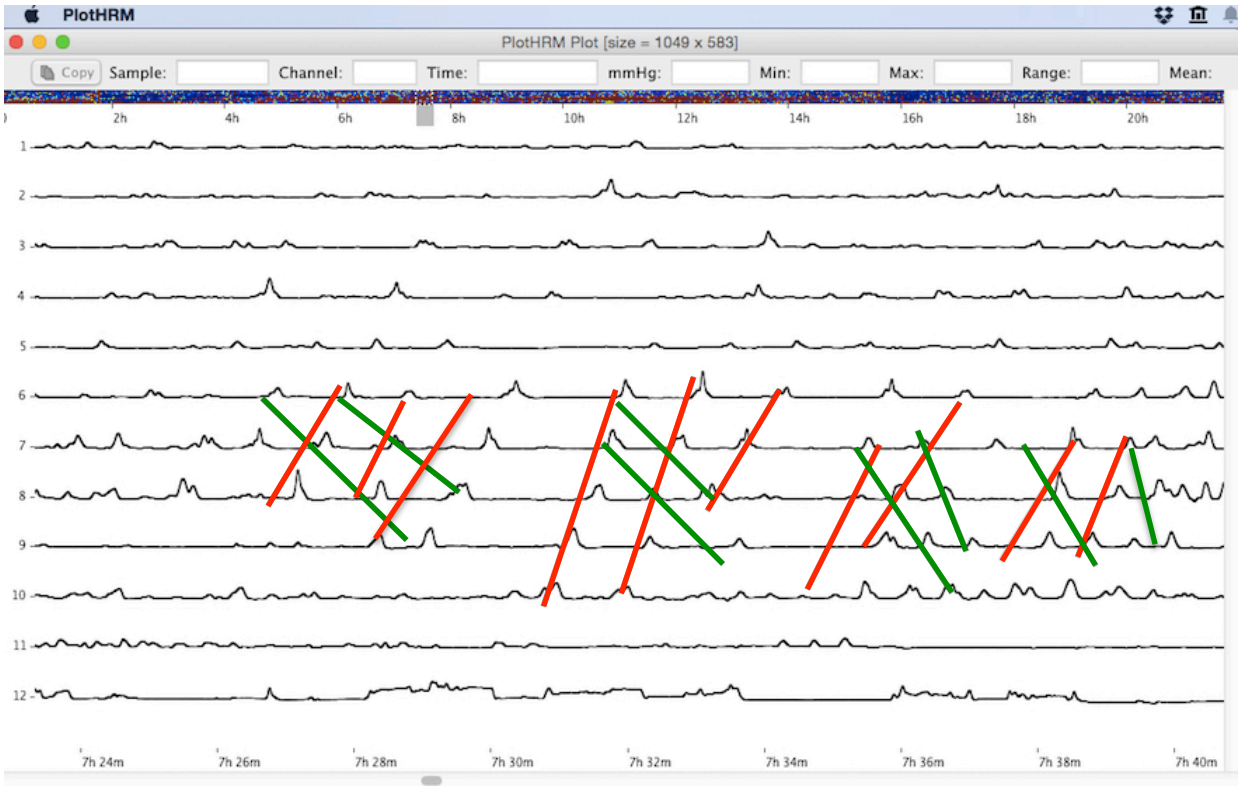


Figure 5.05. Examples of the complexity of colonic motor activities and pressure wave recording during solid-state studies, where it was not possible to accurately confirm propagation and polarity. This complexity was usually more evident with low amplitude rather than high amplitude propagating sequences. The (red line) represents *possible* retrograde propagating motor activity while the (green line) represents *possible* antegrade propagating motor activity. The x-axis represents the time line, while the Y-axis represents the channel position; the top channel is located within the caecal area, while distal channels represent the distal colon (rectosigmoid region).

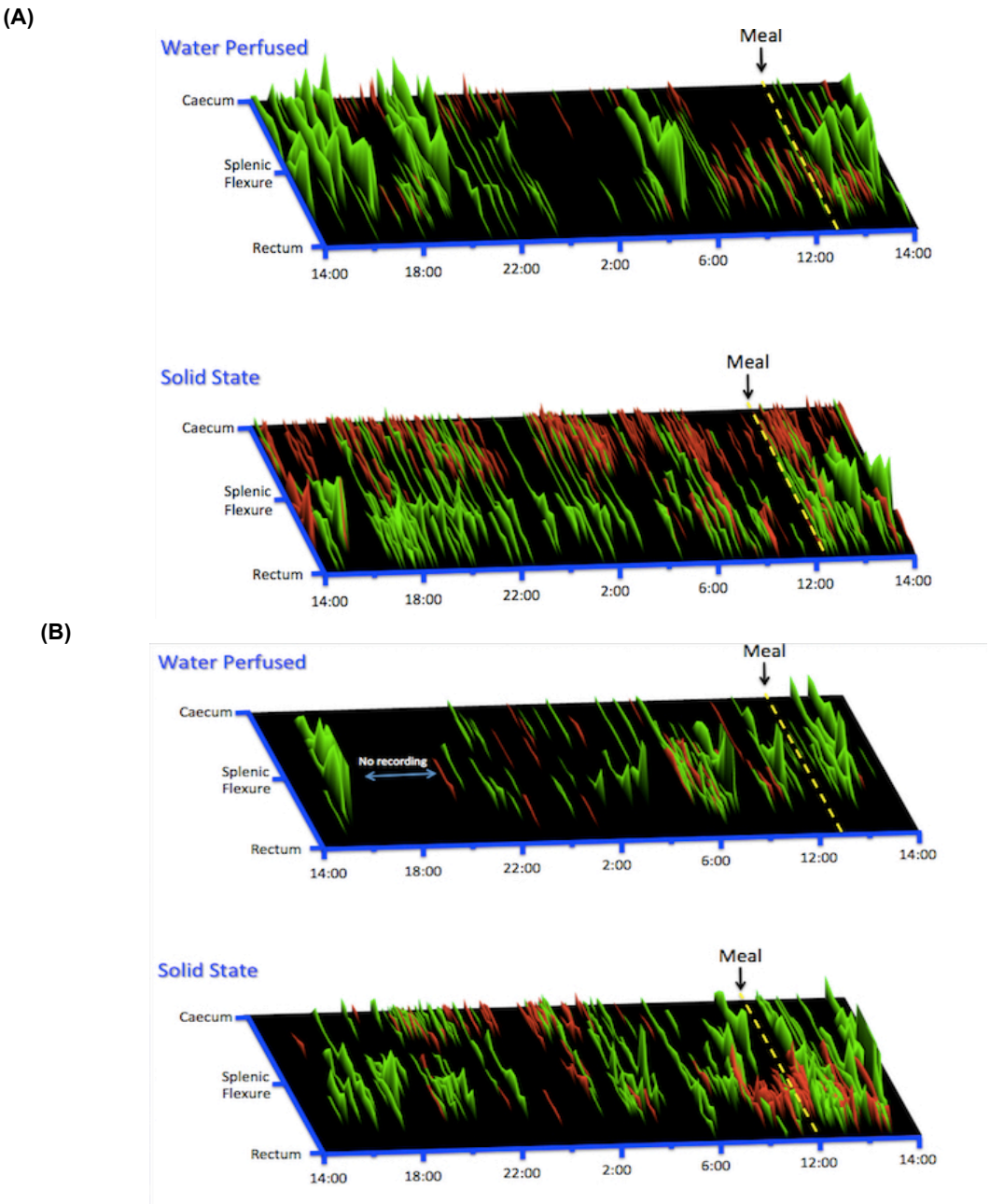


Figure 5.06. **Examples of 24 h spatiotemporal maps in two healthy controls, performed on two different occasions using water-perfused and solid-state studies**. The features immediately apparent include the marked increase in frequency of PS (antegrade and retrograde) in the entire colon using solid-state technology. Additional features include lack of the nocturnal suppression of PS that is observed in water-perfused studies.

5.6.5. QUANTITATIVE ANALYSIS

5.6.5.1. Overall propagating sequences (PS)

Overall, the median amplitude of all identified PS was similar in both groups (whole colon: *P* = 0.6; right colon: *P* = 0.6; left colon: *P* = 0.4) (Table 5.01). By contrast, the overall frequency of PS recorded during solid-state studies was fourfold greater than that detected during water-perfused studies (whole colon: *P* = 0.03; right colon: *P* = 0.01; left colon: *P* = 0.06) (Figure 5.06 and 5.07).

Colonic regions	Whole colon		Right colon		Left colon	
Catheter type	**WP**	**SS**	**WP**	**SS**	**WP**	**SS**
Median PS amplitude (mmHg)	33 (25 - 114)	43 (31 - 54)	39 (25 - 108)	44 (26 - 50)	33 (26 - 128)	51 (39 - 69)
Frequency of PS per 24 h	45 (23 - 156)	175* (161 - 352)	27 (17 - 81)	116* (73 - 282)	13 (6 - 98)	85 (29 - 96)

Table 5.01. Propagating sequence characteristics within the colon. PS = propagating sequence, WP = water-perfused studies, SS = solid-state studies. ***** represent *P* values < 0.05.

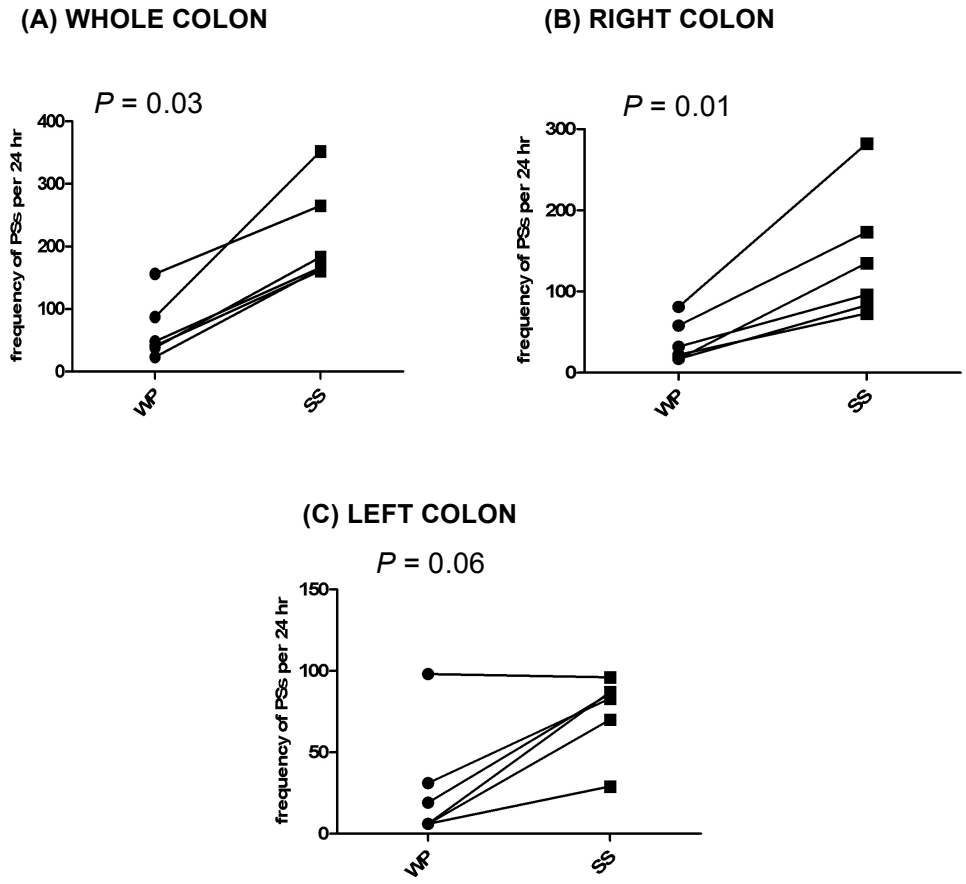


Figure 5.07. Frequency of propagating sequences (PS) per 24 h in water-perfused (WP) and solid-state (SS) studies for each individual subject (n=6), showing significant variability between methods within (A) the whole colon, (B) the right colon, and (C) the left colon.

5.6.5.2. Antegrade propagating sequences (APS)

There was no difference in the median amplitude of APS recorded within the entire colon by the two techniques (whole colon: *P* = 0.6; right colon: *P* = 0.2; left colon: *P* = 0.6) (Table 5.02). Similarly, the overall extent of propagation of the APS within the entire colon and the left colon was similar in both groups (*P* = 0.09 and *P* = 0.5 respectively) (Table 5.02). However, the extent of propagation of the APS within the right colon was longer in water-perfused studies than in the other group (*P* = 0.04) (Table 5.02).

Overall, the frequency of APS within the entire colon and also within the right side, was higher in solid-state studies (*P* = 0.03), but not within the left colon (*P* = 0.2) (Table 5.02).

Colonic regions	Whole colon		Right colon		Left colon	
Cather type	**WP**	**SS**	**WP**	**SS**	**WP**	**SS**
Median amplitude (mmHg)	38 (28 - 88)	49 (34 - 54)	67 (32 - 91)	45 (31 - 52)	41 (0 - 128)	58 (39 - 63)
Frequency per 24 h	28 (23 - 97)	94* (65 - 167)	20 (13 - 59)	63* (23 - 151)	9 (0 - 57)	34 (16 - 52)
Extent of propagation (cm)	23 (23 - 45)	23 (15 - 23)	30* (23 - 45)	23 (15 - 30)	23 (15 - 38)	15 (15 - 23)

Table 5.02. Antegrade propagating sequence characteristics within the entire colon. WP = water-perfused studies, SS = solid-state studies. ***** represent *P* values < 0.05.

5.6.5.3. Retrograde propagating sequences (RPS)

Overall, median amplitude of RPS weas higher in solid-state studies than in other group (whole colon: *P* = 0.06; right colon: 0.09, left colon: *P* <0.03) (Table 5.03).

The overall frequency of RPS within the whole colon and in the right colon was significantly higher in solid-state studies (*P* = 0.03) (Table 5.3) (Figure 5.08). Similarly, the frequency of RPS within the left colon was higher in solid-state studies but this did not reach statistical significance (*P* = 0.09) (Table 5.03). By contrast, the extent of propagation of RPS was similar in both groups and in all colonic regions (whole colon: *P* = 1.0, right colon: *P* = 1.0, left colon: *P* = 0.4) (Table 5.03).

Colonic regions	Overall		Right colon		Left colon	
Cather type	**WP**	**SS**	**WP**	**SS**	**WP**	**SS**
Median amplitude (mmHg)	26 (17 - 34)	43 (26 -56)	24 (0 - 37)	43 (22 - 57)	30 (22 - 46)	50* (34 - 62)
Frequency per 24 h	18 (0 - 59)	94* (65 - 185)	7 (0 - 22)	67* (33 - 131)	7 (0 - 41)	39 (4 - 54)
Extent of propagation (cm)	15 (15 - 23)	15 (15 - 23)	15 (15 -23)	15 (15 - 23)	15 (15 - 30)	15 (15 -23)

Table 5.03. Retrograde propagating sequence characteristics within the entire colon. WP = water-perfused studies, SS = solid-state studies. ***** represent *P* values < 0.05.

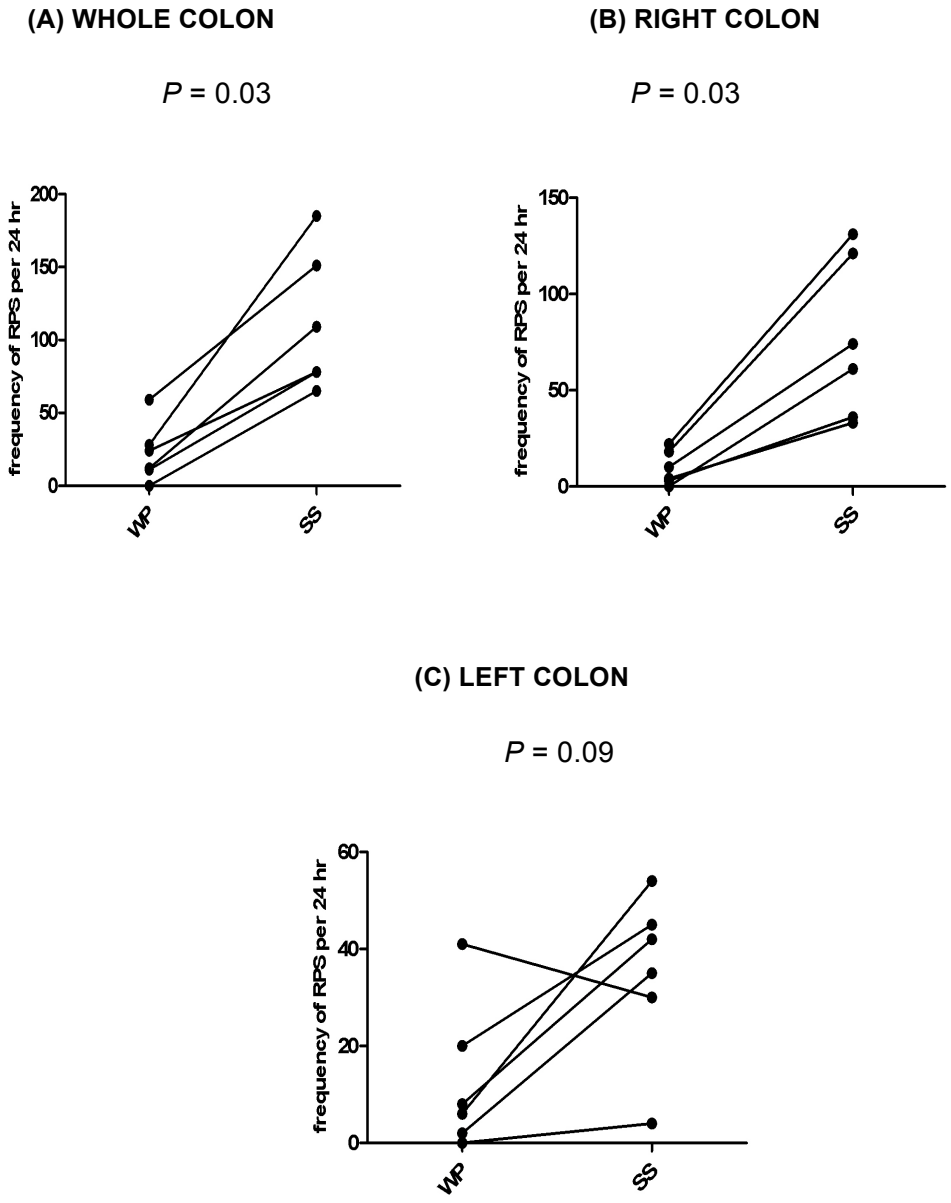


Figure 5.08. Frequency of retrograde propagating sequences (RPS) per 24 h in water-perfused (WP) and solid-state (SS) studies for each individual subject (n= 6), showing significant variability between methods (A) within the entire colon, (B) within the right colon, and (C) within the left colon.

5.6.5.4. High amplitude propagating sequences (HAPS)

The frequency of HAPS recorded in solid-state studies was higher in all colonic regions, though this did not reach statistical significance (*P* value: overall = 0.06, right colon = 0.2, left colon = 0.06) (Table 5.04). Conversely, the overall amplitude of HAPS in all colonic regions was generally higher in water-perfused studies compared to solid-state studies (overall: *P* = 0.06; right colon: *P* = 0.05; left colon: *P* = 0.1) (Table 5.04). The overall propagation distance of HAPS was similar in both groups (whole colon: *P* = 0.2; right colon: *P* = 0.9; left colon: *P* = 0.8) (Table 5.04).

In terms of polarity of HAPS, the majority were antegrade in direction in both groups. The frequency of antegrade HAPS was significantly higher in solid-state than in water-perfused studies (26 [10 - 44) vs.15 [7 - 29] per 24 h respectively; *P* = 0.03), as was the frequency of retrograde HAPS (10 [2 - 32] vs. 0 [0 - 3] per 24 h respectively; *P* = 0.03).

Colonic regions	**Overall**		**Right colon**		**Left colon**	
Cather type	**WP**	**SS**	**WP**	**SS**	**WP**	**SS**
Median amplitude (mmHg)	131 (118 - 141)	94 (87 - 124)	126 (92 - 141)	87 (45 - 129)	136 (94 - 195)	97 (74 - 103)
Frequency per h	0.6 (0.3 - 1.3)	1.4 (0.5 - 3.0)	0.5 (0.1 - 1)	1.0 (0.1 -2.1)	0.2 (0 - 0.3)	0.7 (0.1 - 1.5)
Extent of propagation (cm)	45 (23 - 83)	30 (23 - 38)	53 (38 - 60)	45 (23 - 90)	23 (15 - 53)	23 (15 - 30)

Table (5.04): High amplitude propagating sequence characteristics within the entire colon regardless of polarity. WP = water-perfused studies, SS = solid-state studies. ***** represent *P* values = 0.05.

5.6.5.5. Colonic meal response

In both groups, a 1000 kcal meal induced a significant increase in HAPS frequency compared to the basal period immediately preceding it (solid-state: 1 ± 0.9 per h vs.

3.8 ± 1 per h; *P* = 0.03; water-perfused: 0.2 ± 0.4 per h vs. 2.7 ± 1.8 per h; *P* = 0.03). Comparison of the delta values (HAPS basal – HAPS postprandial) showed no difference between the two groups (*P* = 0.5), indicating that the meal response was similar between the groups. The increase in HAPS was not specific to any particular 30 or 60 min epoch postprandially.

5.6.5.6. Diurnal variation in propagating sequences frequency

In all solid-state studies, there was significant loss of circadian rhythm of colonic motor activities (i.e. loss of nocturnal suppression), as described previously (qualitative assessment, section 5.6.4). This manifested as similar recorded frequencies of PS during the daytime and nocturnal periods (daytime: 8.7 ± 2 PS per h vs. nocturnal: 8 ± 4 PS per h; *P* = 0.6 (Figure 5.09). Indeed, in one subject, PS were significantly more frequent during the nocturnal period (daytime: 8.8 PS per h vs. nocturnal: 14.4 PS per h within the 8 h epoch). Conversely, suppression of PS at night was observed in all water-perfused studies, represented by a significant decrease in the frequency of PS during the nocturnal period (daytime: 3.6 ± 1.7 PS per h vs. nocturnal: 1.6 ± 1.8 PS per h; *P* = 0.03) (Figure 5.09).

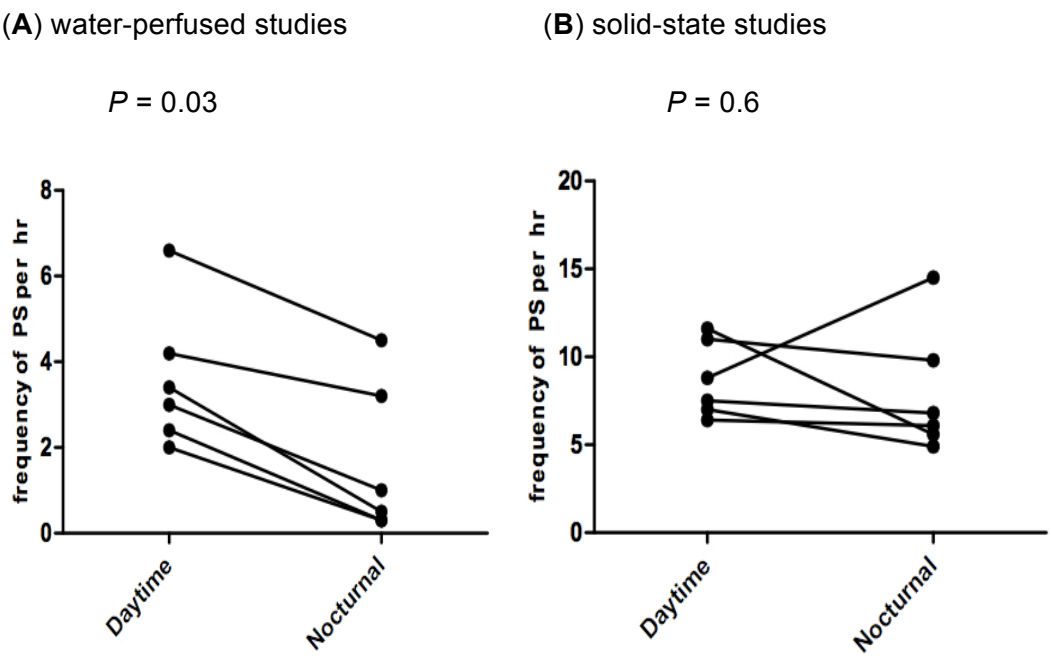


Figure 5.09. Diurnal variation of propagating sequences (PS). Frequency of PS per h during daytime and nocturnal period (8 h epoch each) for each individual subject (n= 6) in (**A**) water-perfused studies: display significant nocturnal suppression and (**B**) solid-state studies: display loss of nocturnal suppression.

5.6.5.7. Frequency of defaecation

No subject defaecated during a solid-state catheter study. One subject did expel a small amount of watery stool following colonic intubation, though not during the recording period. When extubated, all solid-state catheters were covered with a significant amount of semi-formed stool. Subjects who underwent water-perfused studies experienced frequent defaecation during the daytime period, and all had at least one episode of defeacation during the nocturnal period. Overall, 22 episodes of defaecation (of which most were watery stools) were recorded in the six subjects who underwent water-perfused studies (water perfused: median 4 [3 - 5] vs. 0 in solid-state studies; *P* = 0.002). Nine (41%) of the 22 episodes of defaecation showed a normal stereotypical patterns of PS, as previously described (Bampton et al., 2000) (Chapters 3 and 4).

5.7. DISCUSSION

To date, the vast majority of pancolonic manometric studies in adults have utilised water-perfused catheters placed via colonoscopic assistance; the use of solid-state catheters has mainly been limited to the distal colon as previously described. However, the need for continuous water perfusion, which restricts the study subject to the laboratory, and can instil up to four litres of water into the colon over a 24 h period (Bampton et al., 2001), is a major limiting factor. Although the colon is reported to have the ability to absorb large quantities of fluid a day (Debongnie and Phillips, 1978), the effect of introducing such a volume on colonic motility has not formally been investigated. In addition, non-ambulation during water-perfused studies, as subjects need to be continuously linked to the perfusion pump machine, potentially adds another non-physiological confounder to the acquired manometric data. Using a custom-made solid-state catheter, similar in design to the water-perfused catheters used previously (see Chapter 3 and 4), we have investigated pancolonic motility under more 'physiological' conditions (i.e. eliminating the effect of water perfusion) and compared results to those of water-perfused studies within the same subject.

The major finding of this study was the striking overall increase in frequency of PS in both directions (i.e. both antegrade and retrograde) recorded using solid-state catheters. The 'solid-state' technology also appears to influence what has been accepted as a key element of normal circadian rhythm (Narducci et al., 1987, Kumar et al., 1989, Soffer et al., 1989, Bassotti and Morelli, 1990, Bassotti et al., 1993b, Bassotti et al., 1999c, Bampton et al., 2001, Rao et al., 2004) (Chapter 3 and 4), in that the nocturnal suppression of PS was absent when such catheters were used. However, other important elements of normal colonic motility such as the colonic meal response (gastrocolonic response), and exhibition of HAPS were observed similarly using both techniques. Hence, when studying these key features, data derived from different technologies can be compared in a valid way. This is very important as both the colonic meal response and frequency of HAPS are reported to be altered in colonic motility disorders (including slow transit constipation) (see Chapter 1, section 1.6.1.3.1.8). Nevertheless, other parameters that define individual

PS (including amplitude and extent of propagation) in specific colonic regions are influenced by recording technology, which must therefore be taken into consideration when undertaking clinical studies, or comparing with published normative data sets.

To our knowledge, this is the first study that has compared both technologies in an adult population within the entire colon over a 24 h period. The significant increase in frequency of recorded PS using the solid-state catheter is consistent with bench test studies, which showed a faster pressure rise rate with the solid-state catheter (i.e. better fidelity) than the water-perfused catheter. The increase in number of contractions displayed can likely be attributed to the ability of the solid-state sensors to detect and record short-duration pressure changes (i.e. short-duration contractions). A paediatric study comparing both catheter types has shown similar findings both *in vivo* and during bench test studies (Liem et al., 2012).

A second important factor is that, despite intubation technique being equivalent for both catheter types, it appears that the degree of colonic refilling with faecal material is markedly influenced by study methodology; extubated water-perfused catheters were found to be almost clear of faecal residue, whereas solid-state catheters were significantly covered with semi-formed stool. This suggests that solid-state catheter studies were ultimately performed in an environment better mimicking those of the unprepared colon (see Chapter 3). This observation also likely explains the observed reduction in the propagation distance of APS within the right colon, and in the amplitude of RPS within the left colon in solid-state studies. These results are consistent with those shown earlier in Chapter 3, with regional shortening of propagation distance of overall PS and APS within the right colon. Differences compared to water-perfused studies are likely secondary to the effect of continuous colonic loading with water, which acts to ‘wash out’ and dilute right colonic content, with accumulation more distally. Accordingly, frequent episodes of defaecation (mainly liquid stools) were recorded over the study period in water-perfused studies. It is proposed that some of the characteristics of PS recorded in water-perfused studies appear to be ‘augmented’ in the relatively empty colon; in other words, they are ‘damped’ in solid-state studies, where the colon is filled with more viscous

content. Nevertheless, overall amplitude of PS and also APS within the entire colon was similar between groups.

The amplitude and extent of propagation of HAPS were also greater in water-perfused studies compared to those recorded by solid-state studies. However, the frequency of HAPS was similar between groups. This finding is in contrast to that reported by Liem *et al*, who showed that HAPS were detected more frequently with water-perfused catheters, whereas low-amplitude pressure waves were detected more frequently with solid-state catheters (Liem et al., 2012). The difference in reported frequencies between this study and the current study is likely attributed to the frequent episodes of defaecation, which occurred during our water-perfused catheter studies where 41% of these episodes display normal stereotypical PS (including HAPS). If HAPS associated with defaecation were subtracted from analysis, then HAPS would appear to be detected more commonly in solid-state studies.

The other striking finding of the present study is that solid-state studies display higher frequencies (mainly within the right colon) of RPS, and also relatively higher amplitudes of RPS within the entire colon. These findings have never previously been observed in any of our water-perfused studies (see Chapter 3 and 4) nor other prolonged colonic manometric studies in healthy volunteers performed by other groups (Soffer et al., 1989, Bassotti et al., 1999c, Bassotti et al., 1995, Bampton et al., 2000, Bampton et al., 2001, Rao et al., 2001b). This may again simply be a reflection of colonic filling during the entire study period and lack of defaecation, in contrast to water-perfused studies. The obvious difference in frequency of defeacation between groups clearly highlights the effect of continuous water perfusion. Retrograde propagating activities are mainly low amplitude contractions and are poorly described in the literature. However, our previous study (see Chapter 4) showed such activities are mainly identified within the ascending colon of patients with slow transit constipation. In addition, these activities are reported to be present within the entire colon of patients with obstructed defaecation (Dinning et al., 2004). Whether the lack of defaecation during solid-state studies is the primary reason

behind the observed high frequency of RPS merits further investigation. An alternative explanation to the observed increase in RPS activity in solid-state studies may be due to better sensitivity of this type of transducer to detect shorter duration and lower amplitude pressure waves (as described above).

There are some obvious potential criticisms of this study. These include the lack of standardisation of the period between the two studies performed within the same subject. This was mainly due to time constraints allied to difficulties in obtaining regular access to endoscopic facilities in a busy NHS hospital. In addition, the frequent breakage of the solid-state catheters resulted in lengthy periods waiting for repair. Nevertheless, Rao *et al* have reported that findings of prolonged ambulatory colonic manometric studies, including meal response and characteristics of HAPC are generally reproducible within the same subject with some intra- and inter-individual variation (Rao et al., 2010a). However, in that study, the period between recordings was only 2 weeks. Another earlier study performed by Bassotti *et al* assessed HAPC (defined as contractions propagating over two pressure sensors with amplitude >50 mmHg) in three healthy subjects in repeated prolonged manometric studies, and concluded that frequency of these contractions was relatively similar with some intra- and inter-individual differences (Bassotti et al., 1992a). Perhaps the ideal study design is to perform both manometric recordings synchronously (i.e. introduce both catheters into the same subject and record simultaneously), as performed previously by Liem *et al* (Liem et al., 2012). However, this was not deemed possible in the present study due to the difficulties in achieving colonic intubation of both catheters, and presumed subject discomfort, as they would have had to be connected to both recording systems for 48 h, unlike the Liem *et al* study where recording lasted only 3 h. To truly differentiate the effects of water perfusion on recorded colonic pressure activities, paired studies must be performed on two different occasions. Furthermore, the study cohort could be larger and be extended to include patients with slow transit constipation in order to investigate differences, if any, between health and constipation (see Chapter 4). Again, difficulties in securing regular endoscopy sessions limited recruitment. A final consideration was the cost of the solid-state catheter, which was considerable

compared to the cost of replacing the water-perfused catheter. The tendency of solid-state recording channels to break has been previously reported in other colonic manometric studies (Hagger et al., 2002, Hagger et al., 2003), and represents one of the limitations of this technique.

In summary, this study clearly highlights the problems inherent in comparing colonic motor activity data acquired by different technologies and recording catheters. Consequently, investigators should consider the impact of recording technique on parameters that define individual PS (such as amplitude, extent of propagation, and frequency), as well as colonic motor responses to commonly assessed physiological stimuli such as morning waking (but not meal response). In term of practicality, although solid-state catheters theoretically offer a more 'physiological' method by which to record colonic motility, the fragility of the catheters themselves is a limitation. However with the introduction of new fibre-optic technology into the design of colonic manometric catheters (Dinning and Scott, 2011, Dinning et al., 2013, Bampton and Dinning, 2013, Dinning et al., 2014) the possibility of having an alternative to water-perfused studies (which clearly alters the recording environment) is on the not too distant horizon.

6 ACCURATE ASSESSMENT OF COLONIC TRANSIT TIMES USING THE WIRELESS MOTILITY CAPSULE: A VALIDATION STUDY TO LOCATE THE FALL IN PH WITHIN THE ILEOCAECAL REGION USING A DUAL ISOTOPE-SCINTIGRAPHIC TECHNIQUE

6.1. INTRODUCTION

Constipated patients refractory to simple conservative and medical therapies, and in whom organic causes have been excluded, may be considered for referral for further investigation (Cook et al., 2009). At present, on the basis of tests that assess the speed of colonic transit and the efficacy of rectal evacuation, patients with intractable constipation can be broadly subclassified into those with delayed colonic transit (slow transit constipation), a rectal evacuatory disorder, or both (Bharucha, 2007, Cook et al., 2009) (see Chapter 1, section 1.8.6). Such classification is justified, as it has the potential to direct medical (Lembo and Camilleri, 2003), behavioural (Chiarioni et al., 2005, Chiarioni et al., 2006, Rao et al., 1997), and surgical therapies (Knowles et al., 1999a, Nyam et al., 1997). Altered motor activity of the colon can underlie abnormal bowel frequency (Dinning et al., 2009a) (and also evacuatory ability) (Dinning et al., 2004) in a significant proportion of chronically constipated patients, and it is now accepted that the measurement of colonic transit time (CTT) should be the initial test of choice (as an indirect measure of colonic motor function) (Cook et al., 2009, Lembo and Camilleri, 2003). This is presently achieved in clinical practice by two radiological techniques, radioopaque markers and colonic scintigraphy (Dinning et al., 2009a). These methods, however, have limitations; both involve irradiation and there is a lack of standardisation (Dinning et al., 2009a), meaning that results are difficult to compare between centres. Also normative data is lacking (see Chapter 1). Nevertheless, such studies have shown that approximately half of constipated patients will have delayed colonic transit (range 13 - 80%) (Dinning et al., 2009a, Rao et al., 2005). Furthermore, a proportion of these patients have upper gastrointestinal (GI) symptoms, and evidence of a panenteric motor disorder may be found in 18 - 72% (Scott et al., 2003, Zarate et al., 2009). This is of clinical significance, as such patients may have poorer outcome to intervention compared with those patients with an isolated colonic disorder (Redmond et al., 1995). Until recently, only the technique of whole gut scintigraphy has allowed assessment of regional gut transit in clinical practice (Bonapace et al., 2000). However, this test is only available in a handful of specialist centres worldwide. It is

expensive, technically challenging, and, because of the necessity of repeated imaging over long periods of time, is limited by geographical and time constraints for some patients (Dinning et al., 2009a). Colonic manometry is also available in a limited number of institutions offering direct measure of colonic motor function; however, this technique is still not used routinely in clinical practice. GI transit times can alternatively be assessed through the use of ingestible telemetric capsules, which can record biological properties such as pH from within the lumen of the gut (Camilleri et al., 2008, Rao et al., 2009). Although such technology has existed for over 50 years (Connell et al., 1963), it is only recently that commercially produced devices have become available and have been promoted for clinical use (Camilleri et al., 2008). Using pH-sensitive devices, stereotypical changes in pH profile along the GI tract have been demonstrated with two pH 'landmarks' proposed to represent transection zones between specific regions of the gut (Evans et al., 1988, Watson et al., 1972): specifically, a rapid rise in pH from the acidic environment of the stomach to the more alkaline environment of the duodenum is taken as 'pyloric passage'; following on some hours after this, a drop in pH of > 1 unit is thought to reflect movement across the ileocaecal junction (ICJ) from the alkaline terminal ileum to the more acidic proximal colon (Chapter 1, Figure 1.09). Accordingly, these landmarks have been used to differentiate regional GI transit times. However, fundamental to the validity of such a technique is the knowledge of the precise anatomical location of any pH change. The exact location of this fall in pH around the ICJ has, however, never been conclusively substantiated. Previous attempts at validating the position of the capsule relative to the pH profile of the gut were fundamentally imitated by the fact that intraluminal site was derived indirectly through extracorporeal localisation (Bown et al., 1974, Evans et al., 1988, Ewe et al., 1999, Fallingborg et al., 1989, Holdstock et al., 1970, Reynolds et al., 1988, Waller, 1975). Indeed, doubts about the site specificity of the drop in pH around the ICJ have already been raised in the scientific literature (Chourasia and Jain, 2003, Fell, 1996, Nugent et al., 2001), and the exact regional locus of this pH change (ileum, caecum, or colon) remains uncertain (Bown et al., 1974, Fallingborg et al., 1989). If ingestible capsules are to be used as a diagnostic tool to accurately measure regional transit times, then this

information is mandatory.

6.2. AIM OF THE STUDY

Using a dual-scintigraphic technique and the wireless motility capsule (WMC) (SmartPill©; SmartPill Corporation, Buffalo, NY), we aimed to accurately determine the anatomical site of pH change and thus whether this pH change can be used as a biomarker of transition from small to large bowel.

6.3. MATERIALS AND METHODS

6.3.1. STUDY SUBJECTS

Healthy volunteers were identified through advertisement in a similar way to that described previously [Chapter 3, section 3.3.1]. Thirteen healthy volunteers (7 women; median age 29 yr.; range 26 - 53 yr.) participated in the study. Inclusion criteria met those described in Chapter 2 (section 2.3.1). Pregnancy was excluded in all female subjects before enrolment. In all healthy volunteers, no tobacco use was allowed within 8 hours before and after capsule ingestion and no alcohol use 24 hours before capsule ingestion, or during the monitoring period was permitted. All volunteers gave written informed consent prior to enrolment.

6.3.2. EQUIPMENT AND PROCEDURE

6.3.2.1. The WMC (SmartPill) recording system

The system has the ability to provide measurement of pH, pressure, and temperature synchronously (Figure 6.01, 6.02). The capsule is cylindrical, measuring 26.8 x 11.7 mm, and contains a solid-state pressure sensor, an ion-sensing field-effect transducer (ISFET), a pH sensor, a solid-state temperature sensor, electronic subassemblies supporting the pressure and pH sensors, a radio frequency transmitter, and an antenna. The electronic assembly is isolated from the external environment by a rigid polyurethane shell. Two 1.5 V silver oxide batteries, connected in series, power the capsule. The capsule employs a “smart-power control system” to maximise battery life and provides a minimum of 5 days of operational

use, making it ideal for the study of patients with suspected slow bowel transit. pH is accurately measured in the range of 0.5 - 9.0 with an accuracy of +/-0.5 pH unit. The pressure sensor has a pressure range of 0 - 350 mmHg with an accuracy of +/-5 mmHg below 100 mmHg, and 10% at or above 100 mmHg. The temperature sensor has a range of 25 - 49°C, with an accuracy of +/-1°C. Data are transmitted in a serial burst format with each transmission consisting of the sensed pressure value, pH value, temperature, battery voltage, capsule electronic serial number, and a data packet identification number. Each transmission burst contains sensor data acquired during the preceding 20 seconds for the first 24 hours of capsule operation and, subsequently, every 40 seconds for the duration of capsule use. Measurements are transmitted from the capsule within the GI tract at 434 MHz to a specialised patient-worn data receiver (Figure 6.02). The data receiver uses a single integrated antenna and captures the capsule's transmitted data. The sensitivity of that data receiver allows the unit to be worn on a belt, a harness, or placed near the subject. All received data are stored within the data receiver. Upon completion of the study, data are downloaded to a personal computer for analysis.

6.3.2.2. Dual scintigraphic technique

6.3.2.2.1. Radionuclide labelling of the WMC

For the purpose of this study, the WMC was labelled with a radionucleuotide marker to enable localisation of its progression along the GI tract using scintigraphic imaging. Each capsule was labelled with ^{51}chromium[EDTA] (^{51}Cr[EDTA]) (GE Healthcare, Buckinghamshire, UK), which has a half-life of 27.7 days, suitable for prolonged evaluation of colonic transit. Four megabecquerels of ^{51}Cr[EDTA] contained within 1.5 ml of water were injected into a modified polyurethane-polycarbonate blend outer sleeve (Noveon Carbothane PC-3555D) that covered approximately two-thirds of the body of the WMC (Figure 6.01). This was achieved with the aid of a precision pump (Ultra 2400 series; EFD, East Providence, RI, USA) (Figure 6.03). The space between this outer sleeve and the body of the capsule corresponds to a volume of approximately 1.5 ml and can be filled with oil or water,

serving as a closed pressure chamber. The outer sleeve was then sealed using a heat sealer (model no. 70; Clamco, Cleveland, OH, USA). Care was taken to check for leaks; the filled WMC was placed in a 5 ml Falcon tube (BD Biosciences, Oxford, UK) containing water on a stirring plate overnight, and a sample of the water was removed and assessed in a scintillation counter to confirm that no leakage of ^{51}Cr[EDTA] had occurred. If there was evidence of leakage, the capsule was discarded.

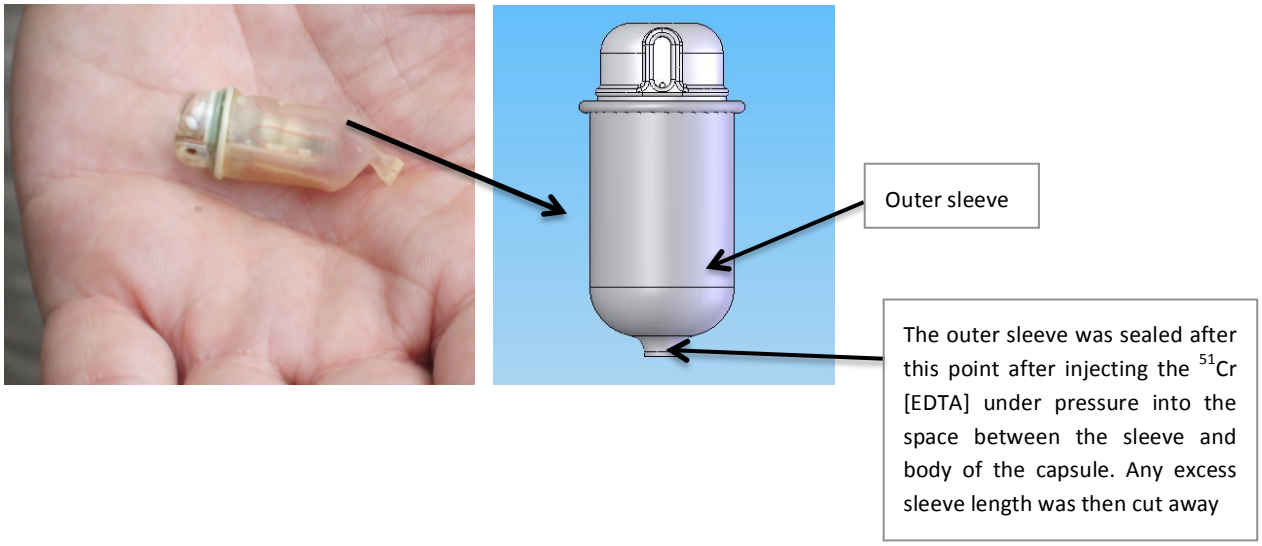


Figure 6.01. The wireless motility capsule. The ^{51}chromium [EDTA] (^{51}Cr[EDTA]) is a radionucleuotide marker contained within the outer sleeve, which has been heat-sealed and test for leakage.

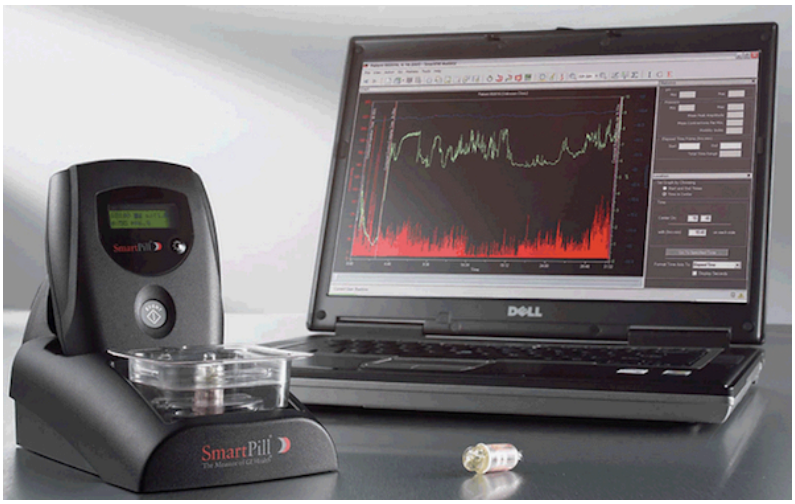


Figure 6.02. The wireless motility monitoring system (SmartPill©; SmartPill Corporation, Buffalo, NY). The wireless recorder was carried in a shoulder harness for the duration of the study or it can be kept close to the subject (for example during the nightime) to enable continuous communication with the capsule.

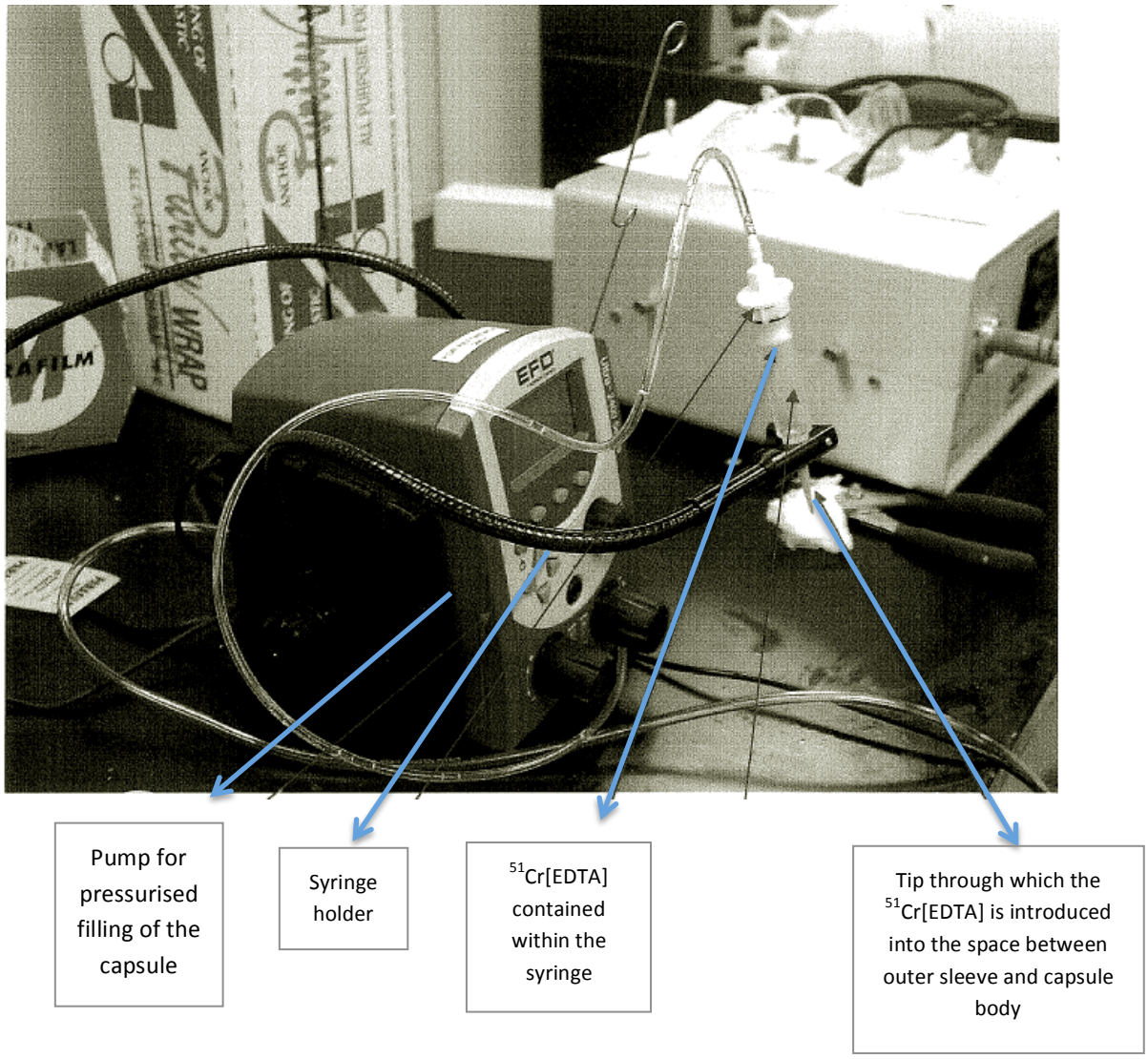


Figure 6.03. Capsule filling. ^{51}Cr[EDTA] was injected under pressure into the space between the outer sleeve and body of the wireless motility capsule. A 2 ml syringe was connected to pressure-operated volume dispenser in order to minimise spilling and accurately introduce the correct volume of 1.5 ml ^{51}Cr[EDTA]. Once introduced, the open end of the outer sleeve sealed with a heater designed for that purpose. The sealing area of the heater was covered with disposable wax paper to avoid permanent contamination of the instrument.

6.3.2.2.2. Intubation technique for providing background labelling of gut anatomy

A customised 3 m long, dual lumen catheter (UniTip: Unisensor AG, Attikon, Switzerland), of outer diameter 3.2 mm, was used for nasoileal intubation in order to deliver a background of ^{111}indium [diethylenetriamine penta-acetic acid] (^{111}In[DTPA]), which outlined gut anatomy around the ileocaecal junction. The catheter had tungsten pellets encased in silicone attached to its tip to aid its passage through the pylorus and also an 8 mm diameter (when deflated) balloon that could be inflated with air through one of the lumens to facilitate propulsion along the small bowel. In the early afternoon of day 1, after an overnight fast, the catheter was passed via the nose into the stomach. To minimise discomfort, the subject was offered application of topical (nasal and throat) Lidocaine spray (Astra Pharmaceuticals, Hertfordshire, UK). A guide wire was then introduced through one lumen of the catheter, to facilitate passage of the tip beyond the pylorus under freeze frame (to minimise radiation exposure) fluoroscopy. This procedure took no more than 10 min. Once in the first part of the duodenum, the guidewire was removed and the balloon inflated with 8 ml of air to stimulate intestinal peristalsis. Once the tip was seen to be beyond the ligament of Trietz, the subject was provided with a standardised 750 Kcal meal. Following this, the subject was instructed to self feed the extra catheter length slowly into the stomach at a rate of 10 cm every 30 min. Position of the tip was determined by fluoroscopy every 2 - 3 h. When 1.4 m of the catheter was within the lumen of the gut (determined from outer markings on the catheter), the subject was allowed to go home. They were instructed to introduce the catheter further, to a depth of 1.8 m, before retiring to bed; at this point they were told to extract air from the balloon to leave a residual volume of 3 ml (to avoid retraction). A 750 Kcal evening meal was provided for the subjects to eat at 20:00 and they were instructed to refrain from eating after 21:00 h. Only water and tea were allowed. The subject returned fasted the following morning (day 2), and the catheter tip position was again checked fluoroscopically. The desired location was when the tip approached the right iliac fossa. If required, further introduction of the catheter was performed until the tip was clearly in the terminal ileum. Position in relation to the ileocaecal valve (ideally 20 - 40 cm proximal to this) was also

confirmed fluoroscopically by administering 20 ml of Gastrograffin (Bayer, Berkshire, UK) through the additional lumen.

6.3.2.3. Capsule administration

Once the catheter was satisfactorily positioned, usually around mid-morning of day 2, it was secured to the patient's face with tape, and the balloon was fully deflated to prevent further progression. The WMC was activated via a magnetised activation unit (SmartPill; SmartPill Corporation, Buffalo, NY), and calibrated for pH using buffers of pH 1.0 and 6.0. The subject then swallowed the WMC with around 100 ml of water. pH data were displayed in real time on the portable recorder and monitored continuously. Once the capsule was recognised as exiting the stomach (this occurred after around 1 h in the majority of subjects), indicated by a rise in pH of around 4 U, from pH 1 - 2 (gastric) to pH 5 - 6 (duodenal), the subject was allowed to consume a 750 Kcal standardised meal, which they were encouraged to eat within 10 min. After completion of the meal, a first aliquot of 2 MBq ^{111}In[DTPA], contained in 0.5 ml of water, was administered through the naso-ileal catheter. A further 5 ml of water was then instilled to flush the catheter lumen. The subject was then transferred to a couch under a single-headed gamma camera (NuclineX-Ring/R camera; Mediso, Budapest, Hungary), fitted with medium energy collimator, where an initial single static scan was taken. Anterior 'static' images were initially acquired every 30 min. To aid anatomical identification, and also allow for movement correction during post hoc data analysis, a ^{57}cobalt (^{57}Co) skin marker was taped in place over the xiphoid process. The diffuse spread of the ^{111}In[DTPA] enabled clear delineation of the terminal ileum, caecum, and colon so that a background 'silhouette' of these anatomical regions was obtained. The ^{51}Cr[EDTA] within the WMC was tracked relative to this by overlaying scintigraphic images. When the capsule was seen to cross the midline and progress toward the lower right quadrant, coincident with a stabilisation of pH at around 7.0, indicating location within the ileum, a first 'dynamic' scan (multiple images) was started. This usually occurred around 4 h after the WMC had exited the stomach. During dynamic scanning, the subject was made comfortable and allowed to listen to music via a personal player; the data receiver was placed next to the subject on the couch, and he or she was instructed to move

as little as possible. Once the dynamic scan had commenced, a second aliquot of 2 MBq ^{111}In[DTPA] was administered to supplement identification of the ileocaecal region. Online pH continued to be monitored very closely. The dynamic scan was continued for as long as the subject could tolerate, or 4 h maximum. They were then allowed a 15 min rest period, where they were encouraged to stretch their legs. Once the subject was back under the gamma camera, dynamic scanning recommenced.

6.3.2.4. Image display and acquisition

With subjects in the supine position, both static and dynamic images were acquired on a workstation (XRingR Console; Bartec Technologies, Surrey, UK) using a 128 x 128 matrix and three energy windows (Software Version 6.02c; Bartec Technologies). These windows were set for 1) ^{51}Cr peak at 322 kV, with a 20% window, 2) the higher energy ^{111}In peak at 245 kV, with a 20% window, and 3) another centered at 150 kV with a 50% window to include the lower ^{111}In peak and the ^{57}Co marker. Static images were acquired for 2 min each. Dynamic scans were acquired with the time frame set depending on the subject body habitus at either 45 or 60 s, to a maximum of 240 frames. Dynamic scans progressed ideally until the capsule was seen to be at the hepatic flexure. Of great importance, the clocks of the display software and pH monitor were synchronised.

6.3.2.5. Extubation and capsule excretion

Once the final dynamic scan had been concluded, extubation was performed by gentle traction with prior application of nasal topical anesthesia; this took approximately 15 to 30 min. Subjects were then allowed to go home with the data receiver. They returned the next day (day 3) after they had opened their bowels to check that the WMC had been expelled. Capsule expulsion was confirmed by loss of interpretable data from the pH sensor, a sustained drop in temperature recorded by the temperature sensor, and absence of the ^{51}Cr[EDTA] signal on static imaging. If the capsule had not been expelled, the subject was instructed to return each subsequent morning until excretion had been confirmed. Data were then downloaded from the receiver to separate portable computer for data display and

analysis.

6.3.3.6. Subject Irradiation

Whole body effective dose was 1.8 mSv in total (1.24 mSv for 4 MBq ^{111}In[DTPA]; 0.2 mSv for 4 MBq ^{51}Cr[EDTA]; 0.1 mSv for the ^{57}Co skin marker; and 0.3 mSv for maximum of 12 freeze frames of fluoroscopy). By way of comparison, the average annual background radiation dose in the UK is 2.2 mSv (Hughes et al., 2005).

6.3.3. DATA ANALYSIS

6.3.3.1. Primary data analysis: Capsule location relative to the pH drop

Review of the pH data was performed through dedicated display software (MotiliGI, SmartPill). In addition, raw data were exported as an ASCII file to a spreadsheet (Microsoft Office Excel; Microsoft, Mountain View, CA), from which the mean pH value was calculated in 45 or 60 epochs, corresponding to the time-locked acquisition period for each frame of the dynamic scintigraphic scan. Three independent observers then reviewed the dynamic scans frame-by-frame on a Linkmed Sun workstation (using MicasXplus Manual Processing Software, Version 5.2; Bartech Technologies) to assess the precise time of passage of the capsule from the terminal ileum through to the caecum and beyond (Figure 6.04). Each anatomical region was assigned a numerical value (terminal ileum 4, ICJ 5, caecum 6, ascending colon 7, hepatic flexure 8), and the position of the pill during each frame was agreed upon and plotted side by side with the corresponding pH value to accurately determine location of the capsule at the time of onset of pH drop.

In order to corroborate results of frame-by-frame analysis, time-lapse video loops were created to show movement-corrected passage of the capsule through the ICJ (Figure 4.05). Data processing of dynamic scans was performed on a separate Linkmed Sun workstation with Maps (Link Medical, Hampshire, UK). The sum of all energy windows was movement-corrected using the ^{57}Co marker as a reference point. This movement correction was then applied to the associated ^{51}Cr peak image. A composite image was generated from the summed frames, which gave

excellent delineation of anatomy, and regions of interest (ROIs) were drawn around relevant parts of the bowel. These ROIs were copied onto the ^{51}Cr movement-corrected image so that activity corresponding to the ^{51}Cr[EDTA] contained in the WMC was clearly visualised, relative to anatomical location (Figure 6.05). Movement through the ICJ, relative to synchronously recorded pH, could again be plotted frame-by-frame or as a movie sequence (Figure 6.06).

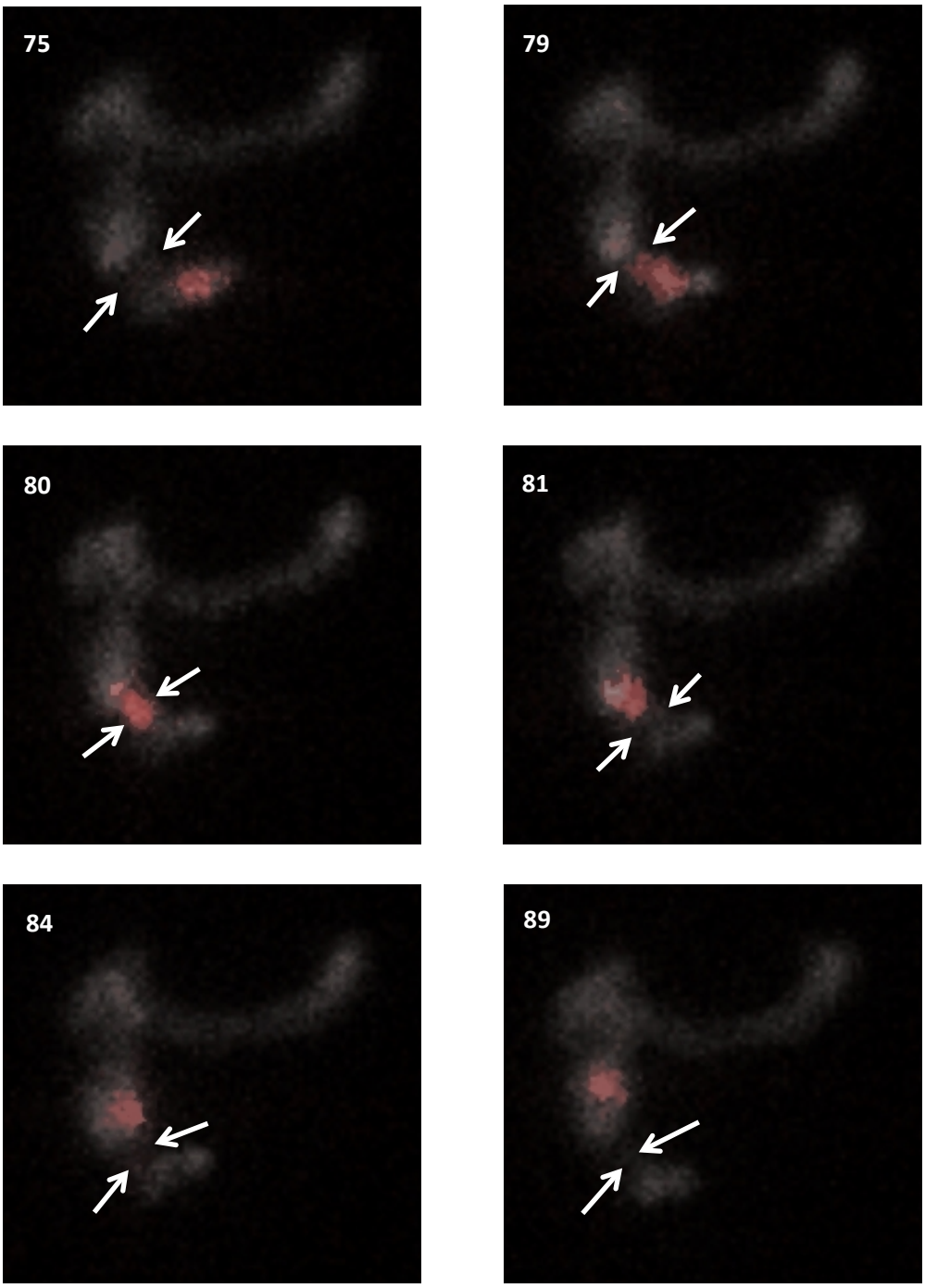


Figure 6.04. Raw images of dual scintigraphy. Position of the ^{51}Cr[EDTA] labelled WMC, relative to the background ^{111}Indium [diethylenetriamine penta-acetic acid] (^{111}In[DTPA]) was determined frame-by-frame (each frame represents an acquisition time of 60 s; frame number shown top left). In this series of images, the WMC (reddish spot) can be seen to pass from the terminal ileum (frames 75 and 79), through the ICJ (frame 80), into the caecum (frames 81 and 84), and eventually into the ascending colon (frame 89). Position of the ICJ is evident in all frames (between white arrows).

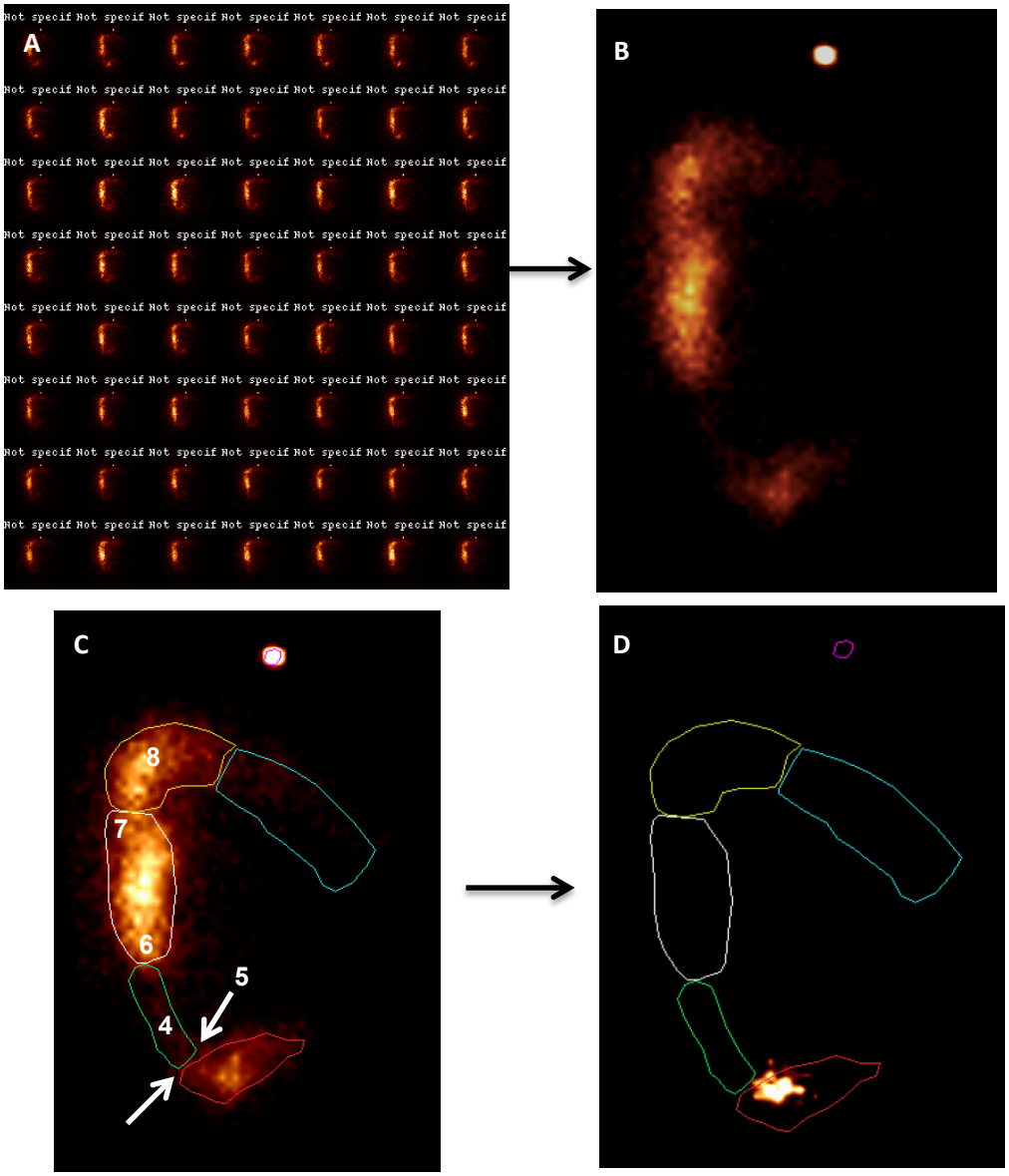


Figure 6.05. Movement correction and creation of regions of interest (ROIs). Individual images (A) were totaled, and movement was corrected using the ^{57}Co skin marker as a reference point (B). ROIs were then drawn around relevant parts of the bowel [C; terminal ileum 4; ICJ 5 (between white arrows); caecum 6; ascending colon 7; hepatic flexure 8], and the activity corresponding to the ^{51}Cr[EDTA] contained in the WMC (bright dot) could be tracked relative to these (D).

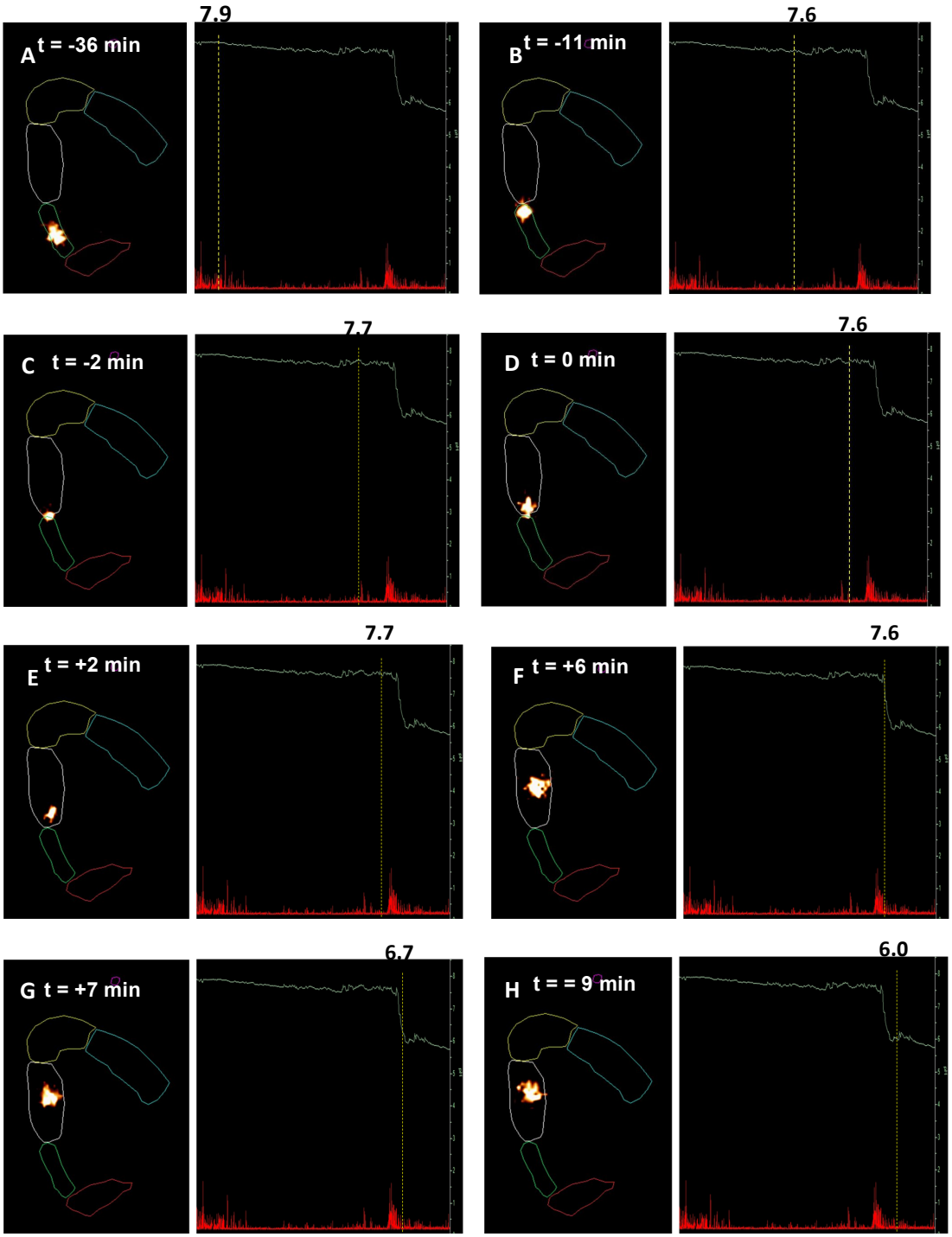


Figure 6.06. Location of the WMC relative to pH change. In this series (A–H), for each displayed time point, the WMC location is shown (left) (see Figure Legend 6.05), corresponding to the pH (green trace) as recorded by the WMC (right). The time locked pH value is represented by a vertical dashed yellow line. Time 0 is taken to be the passage through the ICJ (D). It can be clearly seen that the onset of fall in pH is 6 min after the passage of the WMC into large bowel (F) and occurs within the ascending colon (F - H).

6.3.3.2. Secondary analyses

6.3.3.2.1. Magnitude of pH drop around ICJ

This was defined as the fall in pH from the stable ileal peak to its nadir value. The duration of this fall was also noted.

6.3.3.2.2. Precise location of pH drop

The position of the capsule relative to the ICJ at the start of the pH drop was measured. Approximate spatial resolution was calibrated *ex vivo* by positioning a capsule containing ^{51}Cr[EDTA] under the gamma camera at the usual imaging distance at two sites separated by 10 cm. This allowed calculation of a correction factor for subsequent analysis.

6.3.3.3. GI transit times

Ingestion of the WMC was considered time zero. The time from ingestion to a sustained rise in pH, corresponding to exit of the capsule from the stomach, was considered to represent gastric emptying time (GET). Small-bowel transit time (SBTT) was calculated by subtracting GET from the time of arrival of the capsule in the caecum, as visualised on scintigraphy. CTT was considered the time from arrival at the caecum (as determined scintigraphically) until excretion of the capsule. Whole gut transit time (WGTT) was considered the time from ingestion to the time of excretion. SBTT and CTT were additionally calculated solely on the basis of pH change.

6.3.3.4. Bench validation studies

To determine how rapidly the capsule pH sensor responded to changes in intraluminal pH, a bench simulation study was performed. On 10 successive occasions, the time necessary for the system to record a pH change during transition between fluids of pH equivalent to those around the ICJ was determined. In brief, a WMC calibrated at pH 6 was submerged in 25 ml of Simulated intestinal fluid (without pancreatin) (cat. no. 7109.75; Ricca Chemical, Arlington, TX, USA) at pH 7.54 for 2 min until pH output settled at room temperature. The WMC was then

transferred to a second container of 25 ml of Simulated intestinal fluid (without pancreatin) adjusted to pH 6.36 with approximately 3 ml of simulated gastric fluid (cat. no. 7108; Ricca Chemical). The elapsed time for the capsule to come to a stable reading at 6.36 ± 0.1 pH U was recorded as the response time.

6.3.3.5. Data presentation

All data are expressed as means ± SE for pH, and median and range for time.

6.4. RESULTS

6.4.1. PROCEDURE COMPLICATIONS

Catheter intubation to the terminal ileum or near to the desired area of intubation was successful in most of the subjects (11/13 subjects). Procedure complications during catheter intubation in each subject are summarised bellow in Table 6.01.

Healthy subject no.	Procedure complication
1*	The catheter was extubated because the tip did not progress beyond the pylorus (this subject had a GET of the capsule of 7h)
2*	The catheter was extubated, the balloon that facilitated progression at the tip of the catheter developed a significant leak
3	The balloon that facilitated progression at the tip of the catheter developed a leak. However, in this subject, the tip of the catheter had reached the midjejunum and therefore, the catheter was left *in situ*
4†	The presence of the catheter appeared to impede capsule progression. The WMC was immobile within the duodenum and therefore, the catheter was removed. In this subject, the catheter tip progressed into terminal ileum
5	The presence of the catheter appeared to impede capsule progression. The WMC did not move within the ileum for a period of 2h. Therefore, the catheter was withdrawn 50 cm, and the WMC immediately progressed.
6	No complication
7	No complication
8	No complication
9	No complication
10	No complication
11	No complication
12	No complication
13	No complication

Table 6.01. Summary of procedure complications during catheter intubation. * In these subjects, ^{111}In [DTPA] was given orally in 20 ml water; the first aliquot was given before capsule ingestion, and the second aliquot was administered 30 min after the capsule had exited the stomach. † In this subject, the second aliquot of ^{111}In[DTPA] was given orally immediately after extubation.

6.4.2. IMAGING OF CAPSULE PROGRESSION THROUGH THE ILEOCAECAL REGION

All subjects swallowed the WMC without complication. Capsule progression through the ileocaecal region was accurately assessed in 9/13 subjects. In one subject, the anatomy was impossible to interpret, as ileal loops were overlying the ICJ. In three other subjects, passage of the capsule through the ICJ unfortunately coincided with the rest/exercise period between dynamic scans.

All subjects expelled the capsule within 30 h after oral ingestion. No adverse events occurred except for minor nasopharyngeal discomfort during intubation and extubation.

6.4.3. CAPSULE LOCATION RELATIVE TO THE PH DROP

In all nine subjects where the WMC progression through the ileocaecal region was accurately assessed, a typical pH profile was registered by the WMC pH sensor as the capsule traversed the upper GI tract. Of note, in one of the three subjects in whom ICJ passage was missed, the pH profile was unusual, in that an acute drop of 1.2 pH U was observed during its progress through the jejunum, 80 min after the WMC exited the stomach.

In those subjects included for analysis (n=9), once the WMC was in the terminal ileum, the pH was maintained at a stable value of 7.6 ± 0.05. In 100% of cases (9/9), a drop in pH was observed to occur after the capsule passed through the ICJ and was located in the large bowel (Figure 6.07). Review of dynamic scans revealed episodic bolus flow of ^{111}In[DTPA] through the ICJ, and that capsule progression into the caecum invariably occurred with one of these bolus movements. In five subjects (56%), the onset of fall in pH occurred after arrival of the capsule in the caecum; in two subjects (22%), onset was coincident with a move from the caecum to ascending colon; in the remaining two subjects (22%), onset of pH fall was when the WMC was located in the ascending colon.

At no time were capsules seen to pass back from the caecum into the terminal ileum. However, a transient increase in pH back toward neutral was seen in two subjects following passage into the large bowel; these events occurred at 15 min and 74 min, respectively, after the pH drop associated with small to large bowel transition, and lasted 33 min and 14 min, respectively. In the first case, the WMC was still located in the caecum when this pH rise occurred, and, in the other instance, the capsule was at the hepatic flexure.

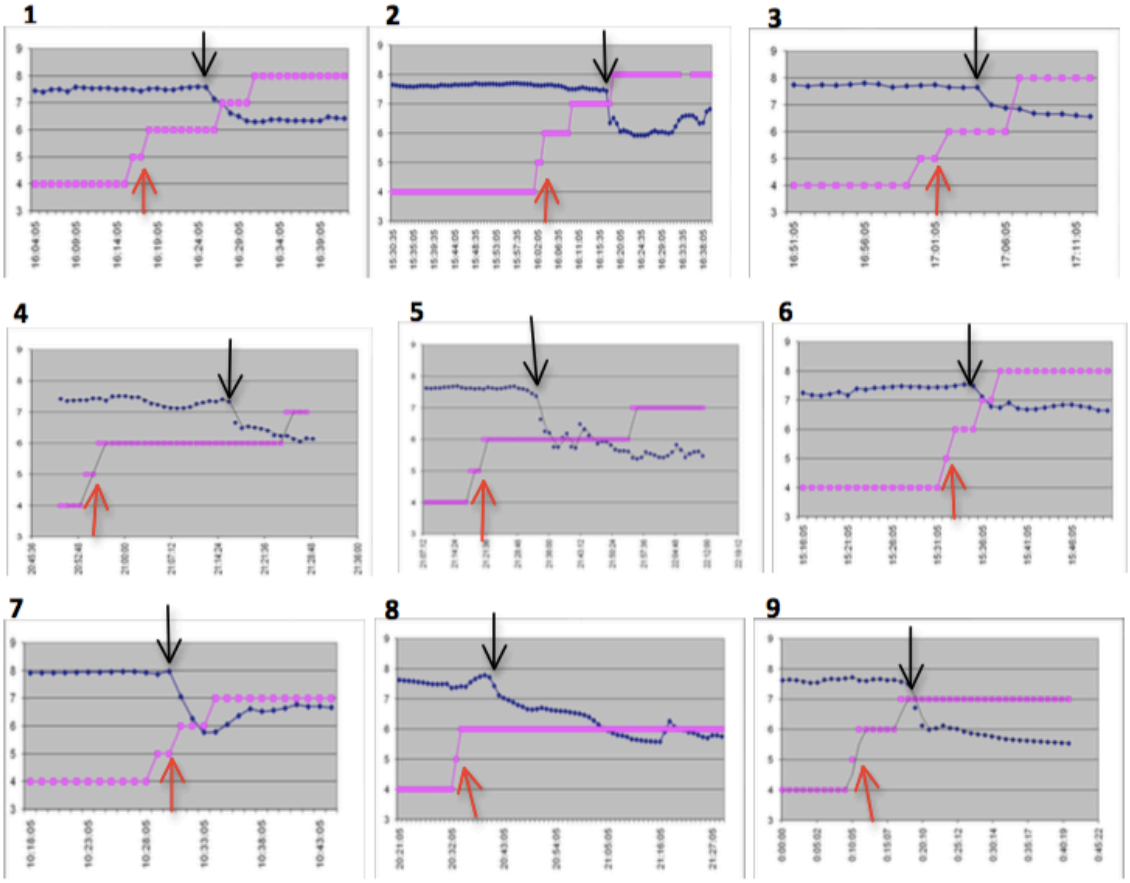


Figure 6.07. Localisation of the pH drop. Synchronous position and pH have been plotted for each individual subject. Time is on the x-axis, and anatomical location is on the y-axis (4 terminal ileum; 5 ICJ; 6 caecum; 7 ascending colon; 8 hepatic flexure). The solid black arrow shows the onset of pH drop. The solid red arrow shows time of passage into the large bowel. In all instances, the pH drop occurred after passage through the ICJ.

6.4.4. MAGNITUDE AND TIME OF PH DROP RELATIVE TO THE ICJ

Overall the magnitude of the pH drop was 1.45 ± 0.20 to a pH value of 6.1 ± 0.1. In those subjects in whom change in pH was noted in the caecum, the fall in pH was 1.35 ± 0.20, compared with those in whom the pH fall occurred in the ascending colon, where the drop was 1.7 ± 0.1. The onset of fall in pH occurred at a median of 7.5 min (range 1 min - 15.45 min) after its passage through the ICJ into the caecum. Nadir in pH was reached at 25 min (3 - 62 min) after arrival into the large bowel. The fall in pH from stable level to nadir was more rapid (median 4 min) in those in whom the drop occurred in the ascending colon, compared with those where the drop occurred in the caecum (median 21 min).

6.4.5. PRECISE LOCATION OF PH DROP

The position of the capsule at the time of onset of pH fall was calculated to be 3 cm (range 1 - 9 cm) distal to the ICJ (though it is acknowledged that this is a 2D measurement in a 3D system).

6.4.6. GI TRANSIT TIMES

GET was 61 min (12 - 337 min). On the basis of scintigraphic confirmation of passage of the WMC into the caecum, SBTT was 342 min (162 - 669 min). CTT, from scintigraphically confirmed arrival in the caecum to excretion, was 723 min (310 - 1047 min). WGTT was 1218 min (537 - 1706 min). If pH change alone was used as a surrogate marker, SBTT was 350 min (169 - 676 min), and CTT was 715 min (288 - 1045 min).

6.4.7. BENCH STUDIES

Median time to record a drop after transition to pH 6.3 from pH 7.54 was 14 s (range 9 - 17 s).

6.5. DISCUSSION

Delayed gut transit is a frequent finding in patients suffering from functional GI disorders including chronic constipation (Balan et al., 2010, Bonapace et al., 2000, Charles et al., 1995); such a delay may involve one or more regions of the GI tract, and hence assessment of regional gut transit is of fundamental importance (Lin et al., 2005). Although radioopaque marker studies are accepted as the 'reference standard' methods for evaluating whole gut transit time (WGTT) (Dinning et al., 2009a, Rao et al., 2005), and are available worldwide thus, do not differentiate patients with localised or a generalised delay in gut transit. The other available method is whole gut scintigraphy, but this also has its limitation (Chapter 1, section 1.6.1.2.2).

Earlier studies reported the ability of indigestible capsules to track pH changes along the GI tract (Watson et al., 1972, Evans et al., 1988), and proposed that such changes can be used as a minimally invasive, non-radiological method to determine regional gut transit. With the recent commercialisation of the wireless motility capsule (WMC), interest in indigestible capsule technology has been revised. Measurements of gastric residence time and WGTT have been validated in studies performed in the USA, using scintigraphic assessment as a 'gold standard' method (Kuo et al., 2008, Maqbool et al., 2009, Rao et al., 2009). However, measurement of small bowel transit and CTT that potentially can be obtained from the same technology has not been validated and depends on accurate localisation of the pH drop around the ICJ.

In the present study, we used simultaneous pH recording derived from the WMC, along with an intensive dual-scintigraphic technique, to confirm the anatomical location for the pH drop in healthy subjects. The major finding of this study is that the pH fall did represent transition from small to large bowel however, the specific site of pH drop varied; in the majority of healthy subjects the change occurred in the caecum, but in a proportion (44%) the fall in pH was determined within the ascending colon. The study also showed that the magnitude of pH drop during capsule transition from the small bowel to the large bowel is approximately 1.5 units; the drop

was more gradual in nature when the drop occurred within the caecum, while a more abrupt drop started occurred when it started within the ascending colon.

The change of pH around the ileocaecal junction was first shown over four decades ago by workers at St. Bartholomew's Hospital, London (Watson et al., 1972). This finding was reproduced by various groups using different ingestible pH-sensitive radiotelemetry capsules (Bown et al., 1974, Evans et al., 1988, Ewe et al., 1999, Fallingborg et al., 1989, Press et al., 1998). In 1988, Evans *et al* (Evans et al., 1988) measured GI pH in healthy subjects, and showed that the mean pH in the terminal ileum was 7.5 ± 0.4, and in all subjects studied (n= 64) there was a sharp fall in pH to a mean of 6.4 ± 0.4 as the capsule presumably passed into the caecum. Likewise, Fallingborg *et al* (Fallingborg et al., 1989) reported that pH gradually increased in the small intestine from pH 6 to about pH 7.4 in the terminal ileum. The pH then dropped to 5.7 after transition into the large bowel. These findings are consistent with the reported pH values in the current study. More recent multicentre trials using the WMC have shown that this decrease in pH is observed in 85% of healthy subjects and also in patients with constipation (Rao et al., 2009). However, attribution of the site of pH drop to the passage of the ingested capsule through the ICJ is flawed by the methods previously used to validate capsule localisation as all studies have significant technical limitations. Those previous methods include position of maximum signal strength emitted from the ingested capsule (Bown et al., 1974, Evans et al., 1988, Thorburn et al., 1992), fluoroscopic imaging (Farthing and Lennard-jones, 1978), changes in pressure waveform as recorded by the capsule (Holdstock et al., 1970, Reynolds et al., 1988, Thorburn et al., 1992, Waller, 1975), or extracorporeal detection of either a metal sphere (Ewe et al., 1999) or radionuclide attached to the capsule (Holdstock et al., 1970, Reynolds et al., 1988, Waller, 1975). Evans *et al* (Evans et al., 1988), derived the location of the ingested pH-sensitive capsule by dividing a drawing of the subject's abdomen into nine sections and mapping the position of the capsule relative to anatomy at given time points on the basis of where maximum signal strength was recorded by an extracorporeal directional aerial probe. The capsule was judged to be in the caecum when maximum signal strength was in the right iliac fossa. However, precise

anatomical location of the pH drop was unknown. Furthermore, the position of the telemetry capsule was only mapped intermittently and only during the day-time (every 2 - 4 h), and therefore localisation at the exact time of the pH drop was likely frequently missed. Moreover, loss of signal was reported from the telemetric capsule over a median 20% of the overall recording time (and up to 64%), meaning that the pH drop itself may not have been captured. Other studies similarly failed to prove the true anatomical location of such drop and were limited to conclude that the drop occurred within the right iliac fossa (Ewe et al., 1999, Holdstock et al., 1970, Reynolds et al., 1988, Waller, 1975). In a study performed by Fallingborg *et al* (Fallingborg et al., 1989) fluoroscopic assessment was used in an attempt to provide a better appreciation to the gut anatomy. However, fluoroscopic imaging was only performed intermittently (every 30 min), and resulted in loss of pH determination in a proportion of studied subjects. Indeed, the authors commented that [we] “cannot rule out that in some subjects we might have misjudged the location of the capsule in relation to the ileocaecal valve” (Fallingborg et al., 1989). Furthermore, the study also used maximum fluoroscopy time of 640 s, which would be considered unethical by today’s standards. A few years later, Thorburn *et al* adopted changes in contractile activity (as recorded by a pressure sensor within the used capsule) to determine the pH drop location. However, the authors concluded that “exact entry into the large bowel was difficult to determine as changes in waveform through the ileocaecal valve are gradual rather than abrupt” (Thorburn et al., 1992).

In the present study, the methodology employed has the advantage over previous techniques because the anatomy was accurately delineated through the use of a background marker (^{111}In[DTPA]), and that localisation was performed relative to this. Furthermore, delivery of the isotopes was generally controlled, with release of background indium being performed near the desired location. In addition, synchronous assessment of capsule location and pH change was performed in real time, and not intermittently as in previous studies (Evans et al., 1988, Fallingborg et al., 1989).

The current study adopted a complex and technically challenging method as we

were primarily looking to capture an event (localisation of the capsule at the precise time of pH drop), which took only a few minutes within a study lasting the greater part of two days. However, this study is not without limitation. In some subjects, indium had to be delivered orally rather than through the ileal tube; however, in these subjects, we were still able to clearly observe the regions of interest. In addition, although the drop in pH around the ICJ region was recorded in all cases, the exact location of the capsule at this point was unfortunately missed in three subjects because it coincided with a resting / exercise period between dynamic scans and that was not avoidable. Interestingly, it appeared that a few minutes of exercise in these subjects was sufficient to promote capsule progression through the ICJ. In all three subjects, the capsule had sat at the ICJ for up to 2 h without significant movement, as determined by dynamic imaging. Finally, in one subject, anatomy was not clear enough due to intestinal looping to allow a confident determination of the location of the capsule. In all other subjects, however, capsule localisation was consistently agreed upon by independent observers.

The bench test performed in this study proved that a pH fall occurred within a few seconds when the capsule was transferred between two viscous liquids with different pH. This suggests that the lag to pH change *in vivo* is a real phenomenon and the recorded timing of the fall in pH was unlikely to be delayed as a result of ileal chyme surrounding the pH sensor of WMC after passage into the caecum. Furthermore, the recording frequency used in this study also makes it likely that the variation in site of pH drop (caecum or right colon) is real.

Other observation of this study were differences in onset of pH change (i.e. gradual or abrupt) and also varying time to reach the lowest pH value (nadir) within the right colon. This likely reflects a pH gradient within the right colon, relative to WMC capsule progression (Cummings and Macfarlane, 1991, Duncan et al., 2009, Macfarlane et al., 1992), and also buffering from terminal ileal contents. The change in pH environment from the small bowel to the large bowel is attributed to the complex human microbiota (Young and Schmidt, 2008), although an individuals' microbiota appears to be quite stable over time (McOrist et al., 2008). The

breakdown of protein, carbohydrates, and nondigestible fibres occurs mainly within the proximal colon. This process of fermentation occurs with the aid of anaerobic bacteria and leads to the production of short-chain fatty acids (SCFA) (Cummings and Macfarlane, 1991, Macfarlane et al., 1992, Bown et al., 1974). However, the biology of SCFA metabolism throughout the colon is poorly understood and is technically difficult to assess directly *in vivo* (Mortensen et al., 1990). Segmental intra-colonic pH is therefore proposed as a surrogate marker of SCFA concentration within a specific colonic region (Mortensen et al., 1990). In the present study, we were able to accurately correlate pH changes recorded by the capsule with exact anatomical location in the proximal colon. Such information may provide clinically important information regarding the concentration of SCFA in relation to common functional GI symptoms (i.e. bloating) (Farmer et al., 2014).

However, a characteristic sharp drop in pH around ICJ region may be absent in up to 15% of studied subjects. This may be attributable to ileocaecal valve competence (Kumar and Phillips, 1987), dietary habits, natural variation of colonic bacterial populations and/ or low SCFA production in certain subjects. For example, Brinkworth *et al*. (Brinkworth et al., 2009) have shown that a very low fibre diet is associated with a significant reduction in faecal concentration of SCFA, compared with a very high fibre diet; whether this translate to changes in pH within specific colonic regions (caecal, proximal, and/ or distal colon) is unclear and warrants further investigation.

The WMC houses a pressure sensor that records intraluminal pressure change as a surrogate of gut contractions. The duration and maximal frequency of human small and large intestinal phasic contractions differ, and these characteristics could theoretically be used as an alternative measure to identify the arrival of the WMC in the colon. However, variability in the state of contractility within the terminal ileum and ascending colon has made identification of the passage of the WMC through these regions, merely based on changes in waveform alone, extremely challenging (Thorburn et al., 1992). Therefore, the development of analysis software is required that is capable of analysing both individual phasic-contraction duration and dominant

frequency, which may ultimately supplement identification of small to large bowel transition, particularly in those subjects in whom the pH drop is unclear. Nevertheless, what has to be borne in mind is that passage through the ICJ takes only 1 - 2 min, and to truly identify an allied temporal change in contractile parameters (in a system where contraction frequency may be only around 3 per minute) may prove unrealistic.

In summary, this study has conclusively shown that the pH drop around the ileocaecal region can be used as a biomarker of transition from the small to large bowel. Furthermore, this fall in pH can be used clinically to determine regional GI transit times (to within a few minutes). Thus the pH drop occurs approximately 10 minutes after passage into the first part of the colon is clinically irrelevant when considering regional transit times of several hours. Whether the pH drop is consistent in different GI diseases (including chronic constipation), or can be influenced by both intrinsic (e.g. gut flora) and extrinsic factors (e.g., diet or drugs, perhaps importantly antibiotics) is unknown and merits further research. Non-radiological wireless capsule methods that measure pH can now be used in clinical practice to accurately determine both small and large bowel transit times.

7 STUDIES OF COLONIC MOTILITY (TRANSIT TIMES AND CONTRACTILE ACTIVITIES) USING THE WIRELESS MOTILITY CAPSULE: COMPARISON BETWEEN HEALTH AND SLOW TRANSIT CONSTIPATION

7.1. INTRODUCTION

The ingestible wireless motility capsule (WMC: SmartPill Corporation, Buffalo, NY) enables the measurement of both regional and total GI transit times and also gut contractile activities (Camilleri et al., 2008) in a minimally invasive, non-radiological manner, without the subject under study having to attend the clinical facility other than for swallowing the capsule and initiating recording. One further major advantage of this technique is that test protocol may be standardised. In patients presenting with symptoms of chronic and intractable constipation, assessment of colonic physiology is paramount, though some patients are suspected to have a pan-enteric dysmotility (Zarate et al., 2009) and thus assessment of other regions of the GI tract, which is feasible using the WMC, is desirable. However, in order for the WMC to be adapted to wider clinical practice, normal ranges for each of the measures provided by this technology need to be derived from studies in healthy volunteers. As determined previously in Chapter 6, the drop in pH in the very proximal part of the colon represents a landmark signalling transition from the small to the large bowel; accordingly, the measurement of colonic contractile activities and colonic pH profile and also colonic transit time can be determined. However, with regard to the former, only right colonic (caecum, and proximal colon), and distal colonic (rectosigmoid) motility and pH can realistically be determined, given the lack of ability to accurately localise the WMC.

For any useful clinical investigation, the endpoint is the ability to differentiate normality from abnormality; this is entirely dependent upon the robustness of 'normal ranges' available. Unfortunately, for tests of lower GI motility (including colonic transit), such normative data are either lacking or derived from relatively small cohorts of healthy volunteers, whose age and gender distribution may not match the target patient populations (see Chapter 1, Table 1.02). Furthermore, in most cases, methods are not standardised. By way of example, for radioopaque marker studies (the most accepted test of whole gut or colonic transit), more than 10 methods involving administration of a single set of markers, and at least 5 methods in which multiple sets of markers are ingested on subsequent days, have been published

(Dinning et al., 2009a). Likewise, with regard to the recording of colonic motor function using manometric techniques, the maximum number of healthy volunteers studied have only been 16 - 20 (depending on the number of colonic regions studied), and various recording catheters and intubation techniques have been used (Dinning et al., 2009a, Scott, 2003) (see also Chapter 1, Table 1.03).

To date, several studies of healthy volunteers have been performed using the WMC in several research centres. By assimilating all available data, it is therefore possible to produce large normative data sets for several measures of colonic motor function and also the hindgut pH profile.

7.2. STUDY AIMS

7.2.1. PRIMARY AIMS

1. to establish normative data for regional GI and colonic transit times using the WMC in a large cohort of healthy volunteers;
2. to establish normative data for proximal and distal colonic pressure profiles using the WMC;

7.2.2. SECONDARY AIMS

1. as to above, to establish normative pH data around the ICJ;
2. as a pilot study, to compare these measures with results derived from a group of patients presenting with slow transit constipation.

7.3. MATERIALS AND METHODS

7.3.1. STUDY POPULATION

7.3.1.1. Healthy volunteers:

Healthy volunteers who underwent a WMC test during the period March 2005 to November 2011 were included. Two hundred and thirty-one WMC data files of healthy volunteers were collected from two sources:

(**1**) studies performed in the USA; these were supplied by the SmartPill Corporation (Dr Jack Semler, Chief Technology Officer) to our research centre as anonymous coded studies. The data acquired in the USA were primarily derived from 2 published multi-centre clinical trials: (1) data from healthy controls used for a prospective study of gastric emptying in gastroparetic patients (Kuo et al., 2008), performed at seven medical centres in the USA; and (2) data derived from healthy volunteers involved in a trial studying colonic and whole gut transit in constipated patients (Rao et al., 2009); this was performed at five medical centres in the USA.

(**2**) studies carried out in Sweden by Dr Per Hellström; the data for these healthy subjects was provided by Dr Hellström to our research centre as anonymous coded studies.

Healthy subjects from the USA were screened with a validated gastrointestinal (GI) disease questionnaire (Locke et al., 1994), and healthy subjects from Sweden were screened with the ROME questionnaire for detection of functional GI disorders (translated into Swedish) (Drossman and Dumitrascu, 2006) and the Gastrointestinal Symptom Rating Scale (GSRS-IBS) (Svedlund et al., 1988) to exclude those with significant GI symptoms.

All studies were approved by Institutional Review Boards or Ethics Committees at participating sites. All healthy volunteers included in this study however, followed the general inclusion as detailed in chapter 2, section 2.3.1.

7.3.1.2. STC patients

Based on previous studies that established a cut-off point of colonic transit time in healthy controls (Rao et al., 2009, Camilleri et al., 2010), delayed colonic transit was defined as >51 h capsule residence time within the colon. Recordings of 19 patients with STC were obtained from two centres in the UK:

(**1**) patients referred to the GI Physiology Unit Colorectal Service at the Barts Health Trust (Royal London Hospital) for further evaluation of their intractable symptoms of constipation. As part of ethically approved multicentre clinical trial, they were selected on the basis of delayed transit on ROM and underwent WMC studies to establish the agreement of measuring colonic transit times using both WMC and ROM (Camilleri et al., 2010). WMC studies were all performed by the author.

Initially, all referrals were made to Dr Mark Scott (the Unit Director) by surgical or gastroenterological consultants. Consecutive patients with proven delayed colonic transit (as confirmed by ROM studies that were performed as a part of their clinical investigative workup) were invited to participate in the multicentre clinical trial. Invitation letters for participation in the clinical trial were sent to all eligible patients by the author, who had examined their presenting clinical history in detail; this had been reported during their initial clinical visit to the GI Physiology Unit Colorectal Service. Patients who agreed to participate in the study were then invited to attend a screening interview visit prior to their enrolment in the trial. During this visit, appropriate consent was obtained. All patients in the UK participated in the multicentre clinical trial were included in this study;

(**2**) Patients referred to the Functional Gut Clinic London with significant symptoms of chronic constipation (CCCS >15), who underwent a WMC study as a part of their clinical workup. Recordings from these patients were provided as courtesy of Dr Anthony Hobson as anonymised coded studies. These patients had not had a prior ROM study but were included on the basis of a colonic transit time, as measured by WMC, of > 51 h. These subjects also had not undergone lower GI physiology testing (including rectal sensation, assessment of anal sphincters function and rectal

evacuation). Otherwise, all STC patients followed the general inclusion and exclusion criteria described in Chapter 2, section 2.3.2.

In all study subjects, no tobacco use was allowed within 8 hours before and after WMC ingestion and no alcohol use 24 hours before capsule ingestion or during the monitoring period was permitted. All volunteers gave written informed consent prior to enrolment.

7.3.2. WMC AND MONITORING SYSTEM

The WMC (SmartPill Corporation, Buffalo, NY) has been described in detail previously (Chapter 6, Figure 6.01 and 6.02). For all studies, proprietary software (MotiliGI® version 2.2 & GIMS® version 2.2.2. SmartPill Corporation, Buffalo, NY) was used for data display and analyses.

7.3.3. STUDY PROTOCOL

7.3.3.1. Healthy volunteers

All subjects fasted overnight. Two different study protocols were followed:

Protocol 1, subjects ingested the WMC first with 50 ml of water followed by an "Egg Beater" meal, which consisted of a scrambled egg substitute mixed with 1 mCi ^{99m}Tc sulphur-colloid marker (120 g Egg Beater, 60 kcal), two slices of bread (120 kcal), strawberry jam (30 g, 74 kcal), and water (120 mL); total caloric value of 255 kcal (72% carbohydrate, 24% protein, 2% fat and 2% fibre) (Kuo et al., 2008).

Protocol 2, subjects ingested the meal first, which was either the "Egg Beater" meal or an equivalent 262 kcal nutrient cereal bar (SmartBar: SmartPill Corporation, Buffalo, NY), composed of 66% carbohydrate, 17% protein, 2% fat, and 3% fibre, along with 50 mL of water, followed by the WMC (Rao et al., 2009).

After swallowing the WMC, all subjects were observed for at least 6 hours within the study centre. During this period, they were not allowed to eat or sleep. All subjects

were then fed a second standardised meal (250 ml Ensure; Abbott Laboratories, Abbott Park, USA) after 6 hours. Subjects were then allowed to go home and advised to perform their usual activities until the capsule was passed naturally with a bowel movement.

7.3.3.2. STC patients

Patients with STC followed Protocol 2, i.e. ingestion of a standardised test meal (SmartBar; SmartPill Corporation, Buffalo, NY) followed by ingestion of the WMC capsule along with 50 ml of water. Once communication was established between the WMC and data receiver, and the capsule was confirmed to be in the stomach (pH < 4), the patient was instructed in receiver care and allowed to leave the department. No further meals or drinks were allowed for 6 h post capsule ingestion. After this, patients were allowed to eat and drink normally. After each bowel movement, the patient was instructed to wait for 1 min prior to flushing the toilet in order to observe a drop in temperature or signal disconnection and this confirmed exit of the capsule. The patient was instructed to then call the department to be given details on how to turn the receiver off and then return it to the study centre for downloading of recording data.

7.3.4. DATA ANALYSIS

WMC pH profile and transit data for each subject were analysed manually. Equivalent data were also obtained from the automated software (MotiliGI® SmartPill Corporation, version 2.2) and compared with the corresponding manually obtained data, to determine study agreement between the 2 methods. The WMC colonic pressure profiles were obtained using semi-automated proprietary software (GIMS® SmartPill Corporation, version 2.2.2) designed for this purpose. The definitions of study parameters are as follows:

1. Regional transit times were based on clear identification of the following stereotypical landmarks (Figure 7.01):

a) time of capsule ingestion (CI) was identified by an abrupt rise in the recorded temperature and drop in pH (reflecting passage into the acidic environment of the stomach);

b) exit from the stomach (passage through the pylorus: PY) was identified by an abrupt rise in pH of usually more than 2 pH units;

c) passage through the ileocaecal junction (ICJ) was determined by a drop in pH usually of more than 1 pH unit (see Chapter 1), sustained for at least 10 minutes, occurring at least 30 minutes after the capsule had exited the stomach;

d) time of WMC expulsion (CE) was determined by an abrupt drop in temperature followed by a loss in recorded signal after the subject had opened their bowels.

The following transit times could then be determined (Figure 7.01):

a) gastric emptying time (GET): duration between the CI and PY;

b) small bowel transit time (SBTT): duration between the PY and ICJ;

c) colonic transit time (CTT): duration between ICJ and CE;

d) whole gut transit time (WGTT): duration between CI and CE;

Only values for CTT are presented. Transit times for other GI regions were calculated, but have been omitted for brevity.

2. In a smaller subset (n = 54) of healthy volunteers who followed meal protocol 2 and also STC patients, the colonic pressure profile was measured by the following methods:

a) colonic contractile frequency (CCF), defined as the frequency of contractions

above 10 mmHg occurring per minute throughout the colon, as previously determined (from ICJ to CE);

b) colonic motility index (CMI), defined as Ln(sum of amplitude x the number of contractions above 10 mmHg + 1) (from ICJ to CE);

c) proximal colonic contractile activity (PCCF) defined as the frequency of contractions above 10 mmHg in the 60 minutes following ICJ passage;

d) proximal colonic motility index (PCMI) defined as Ln(sum of amplitude x the number of contractions above 10 mmHg + 1) within the 60 minute period following ICJ passage;

e) distal colonic contractile frequency (DCCF) defined as the frequency of (presumed) recto-sigmoid contractions in the 60 minutes before CE;

f) distal colonic motility Index (DCMI) defined as Ln(sum of amplitude x the number of contractions above 10 mmHg + 1) within the 60 minute period prior to CE.

Other GI regional pressure profiles were also analysed in a similar fashion; however, they have been, omitted as they are beyond the scope of this thesis.

3. Delta ICJ pH values determined by subtraction of the median caecal pH value in the first 15 minutes after capsule passage through ICJ from the median ileal pH value in the final 15 minutes before capsule passage through the ICJ.

Although pH values from all other parts of the GI tract were also assessed, these data are also omitted as they are beyond the scope of this thesis.

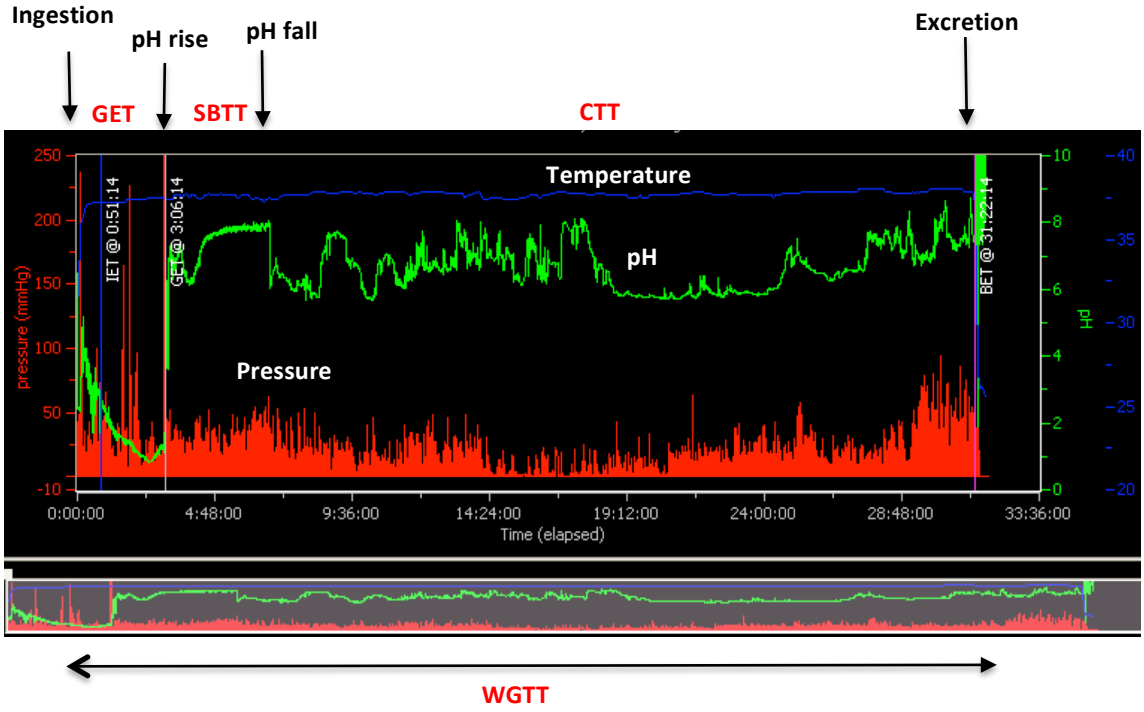


Figure 7.01. Typical wireless motility capsule (WMC) recording. A 32.5 h compressed trace is shown. Phasic pressure activity is shown in red (mmHg, *y*-axis to left), pH profile in green (pH U, *y*-axis to right), and temperature in blue. Regional gastrointestinal (GI) transit can be determined on the basis of 2 characteristic changes in pH recorded by the WMC, namely a rise in pH when it exits the stomach, and a fall in pH where it is known to passes through the ileocaecal junction (ICJ). This allows calculation of gastric emptying time (GET), small-bowel transit time (SBTT), and colonic transit time (CTT). Whole gut transit time (WGTT) is the time between ingestion and excretion.

7.4. STATISTICAL ANALYSIS

Primary study parameters were CTT, and overall colonic, proximal and distal colonic pressure activity profiles.

Secondary parameter was delta ICJ pH. Effects of age, gender and meal protocol on parameters obtained were examined.

Statistical methods

The primary and secondary parameters were summarised using a number of observations, mean and standard deviation. To assess the impact of age, gender and meal protocol on the primary and secondary parameters, a multiple linear regression model was employed. To compare the agreement between automated software analysis and the manual reading of primary parameters, a mixed model was used to estimate the intra-class correlation coefficient (ICC). The mixed model included age, gender and meal protocol as fixed effects, and subject as a random effect and was interpreted as per Yen *et al* (Yen and Lo, 2002). A higher ICC, close to >0.7, suggests good agreement between the two types of readings, whereas a value <0.4 indicates poor agreement. All statistical analyses were performed using SAS 9.2. (SAS institute Inc, Cary, North Carolina, USA) and also GraphPad Prism (GraphPad software Inc., USA, version 5). Two-tailed tests were used throughout. A *P* value of less than 0.05 was adopted as the statistical criterion.

7.5. RESULTS

7.5.1. STUDY POPULATION AND DEMOGRAPHICS

7.5.1.1. Healthy volunteers

A total of 231 data files were available. Of these, 16 had major signal loss and were excluded from analysis; most of these recordings came from early studies where prototype equipment was used. Of the 215 remaining data files, 40 came from studies performed in Sweden and 185 came from studies performed in the USA. Capsule ingestion was identified in all 215 subjects. CE could not be identified in 21 subjects because the recordings ended prematurely, or a capsule expulsion time could not be clearly defined; in these subjects, CTT could not be determined. Furthermore, ICJ could not be identified in another 12 subjects, so CTT also could not be determined in this group. Overall therefore, CTT would be calculated in 182 subjects. A summary of subjects demographics are shown in Table 7.01A.

A sub-analysis of 54 data files was performed to evaluate colonic pressure profiles (Table 7.01B). All data files were randomly selected from the original database; all subjects had followed meal protocol 2. One had major signal loss at the end of recording and was excluded from distal colonic pressure profile analysis. Five had intermittent signal loss after ICJ exit and therefore no information on overall colonic and proximal colonic motility is provided.

7.5.1.2. STC patients

A total of 19 data files were available. One had major signal loss and was excluded from analysis. Of the 18 remaining data files, 12 came from studies performed at the Functional Gut Clinic and 6 came from studies performed at the Royal London Hospital. Accurate CE could not be identified in two patients because the recordings ended prematurely; accordingly, data for distal colonic pressure profile and pH were based on 16 patients. Overall colonic motility could not be measured in another two patients due to intermittent signal loss after passage through the ICJ, and therefore, pancolonic motility data provided were based on 14 patients. A summary of patients'

demographics are shown in Table 7.01B.

(A)

	Overall		Country			
			USA *		Sweden	
Meal protocol	**1**	**2**	**1**	**2**	**1**	**2**
(n)*	74	106	74	74	0	32
Gender (female: male)	76:92†		58:78		17:15	
Median age (range) ‡	34 (19 - 80)		37 (19 - 80)		23 (19 - 73)	

(B)

	Healthy volunteers (n = 54)	STC patients (n = 18)
Gender (female: male)	25:26*	15:3
Age: median (range)	24 (19 - 68)*	44 (22 - 67)

Table 7.01. Subjects demographics (**A**) healthy volunteers demographics with valid colonic transit time. *2 values missing, †14 values missing, ‡ 16 values missing; (**B**) demographics of subset of healthy volunteers and patients suffering from slow transit constipation (STC) who followed meal protocol 2 and in whom colonic motility data was analysed. (n): number; * 3 values missing.

7.5.2. COLONIC TRANSIT TIME (CTT)

CTTs are presented for the whole group and as subgroups classified by the 2 most significant factors identified from the linear regression analysis, i.e. meal protocol and gender, (Table 7.02).

Notably, WCTT and also CTT showed an interesting clustering of data at values separated by 24 hours, rather than being distributed normally, as has been presented previously (Evans et al., 1992). As shown in Figure 7.02, which represents WGTT and CTT, nearly 50% of CE occurred around 24 hours after capsule ingestion, with a second peak (comprising another 17%) occurring at 48 hours (Figure 7.02). The 95th percentile for CTT derived from healthy volunteers in this study was 51 h (Table 7.02), which was equivalent to the upper limit of normal for CTT as defined in previous studies (Rao et al., 2009, Camilleri et al., 2010).

In STC patients, median CTT was 65 h, range: 60 - 129 h.

Parameter	Meal protocol	Gender	N	Mean	SD	Median	5th percentile	95th percentile
Colonic transit time (h)	All	All	182	23	16	19	3	51
	1	F	30	24	1	18	2	59
		M	43	18	12	16	2	36
	2	F	45	25	14	21	7	50
		M	50	23	16	19	4	51

Table 7.02. Normative data for colonic transit times (hours); **F**: female; **M**: male; **N**: number. **SD**: standard deviation; **h**: hours.

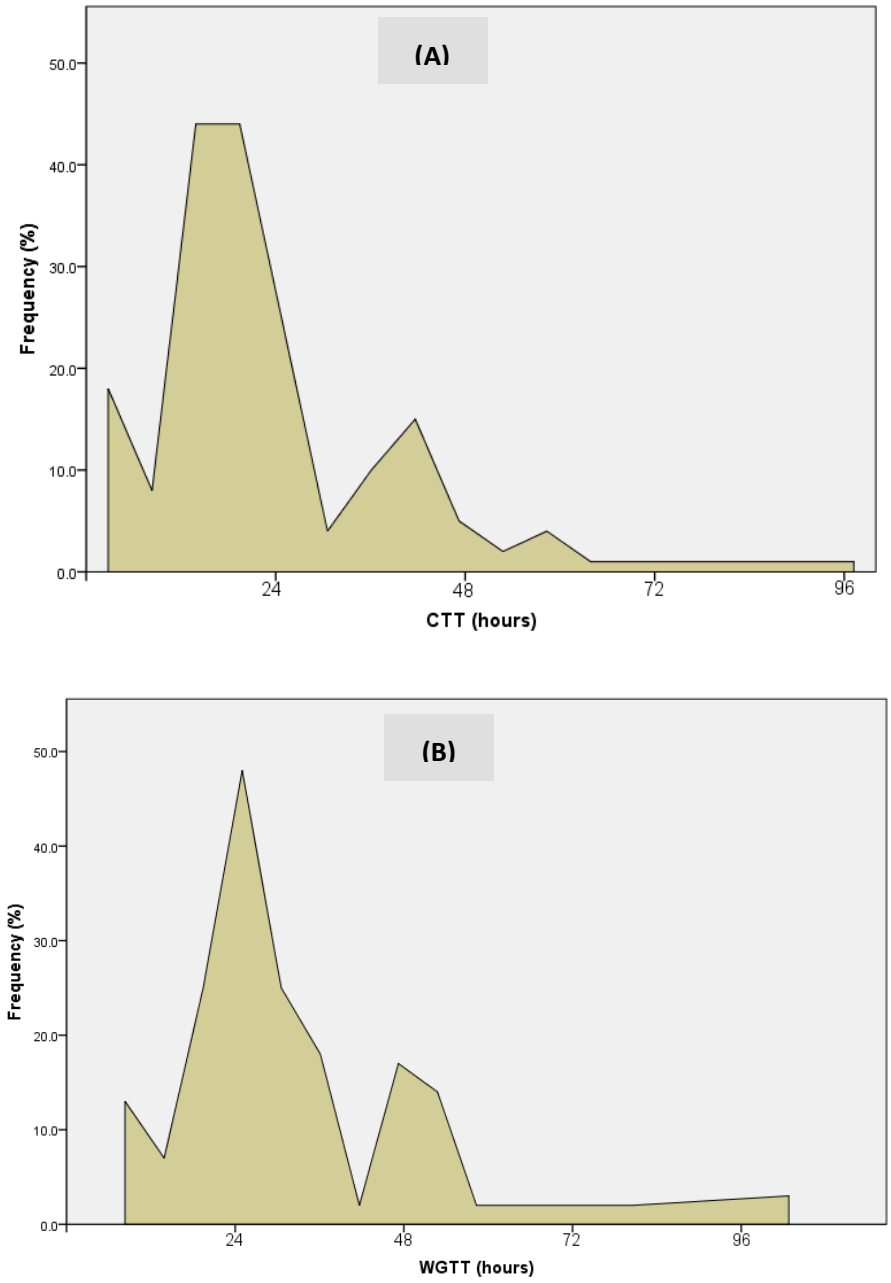


Figure 7.02. Frequency polygon of (A) colonic transit time (CTT) and (B) whole gut transit time (WGTT) in hours in healthy controls. Frequency: percentage of WMCs expelled.

7.5.3. EFFECT OF AGE, GENDER, AND MEAL PROTOCOL ON CTT IN HEALTHY VOLUNTEERS

Linear regression analyses demonstrated that meal protocol was statistically significantly associated with differences in CTT (longer with protocol 2: *P* = 0.015). Females were also shown to have longer CTT (*P* = 0.023). Age did not have any effect on CTT (Table 7:02).

7.5.4. AGREEMENT BETWEEN MANUAL AND AUTOMATED CTT MEASUREMENTS

The agreement between CTT determined manually and those obtained by the automated software, as expressed as intra-class correlation coefficients, was 93%.

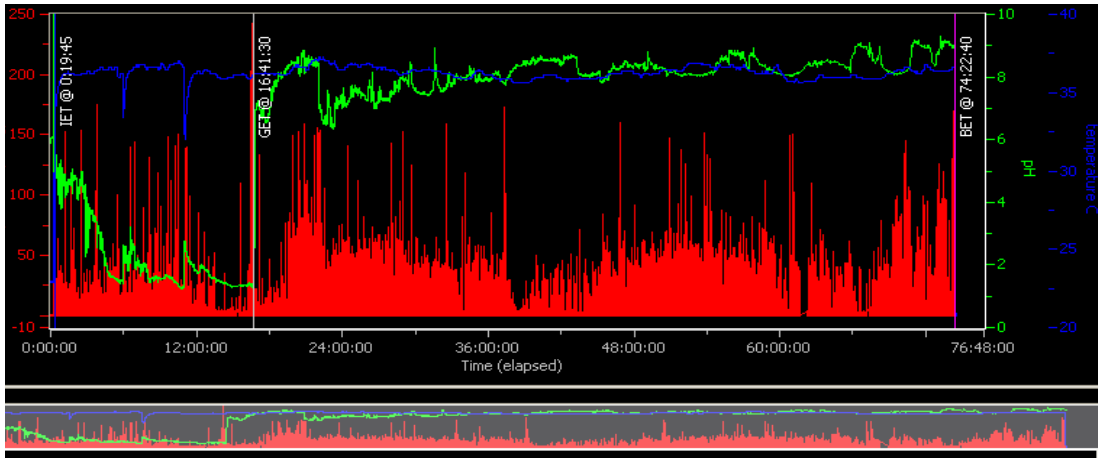
7.5.5. PANCOLONIC PRESSURE PROFILE

Overall pressure profile measurements in healthy volunteers and STC patients are presented in Table 7.03 and in Figure 7.03. CCF did not differ between the two groups (*P* = NS). However, CMI was significantly higher in STC patients than in healthy volunteers (*P*< 0.0001).

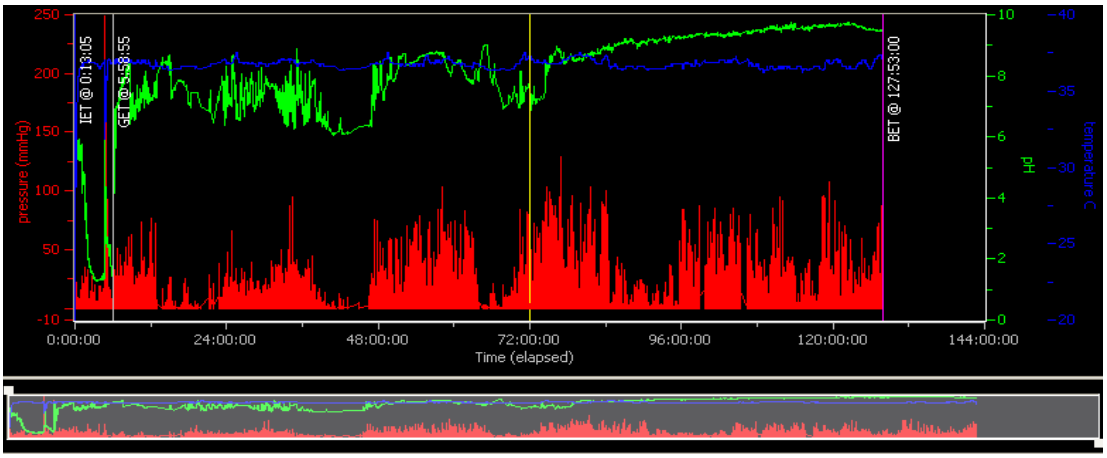
	Group	N	Mean	SD	Min.	Max	*P* value
CCF	normal	49	2.8	1.2	0.6	5.9	0.4
	STC	16	3.0	1.3	1.1	6.6	
CMI	normal	49	184.0	104.0	18.5	460	**<0.001**
	STC	16	330.0	166.2	110	691	

Table 7.03. Overall colonic pressure profiles in healthy volunteers and STC patients; CCF: colonic contraction frequency; CMI: colonic motility index; N: number.

(A)



(B)



(C)

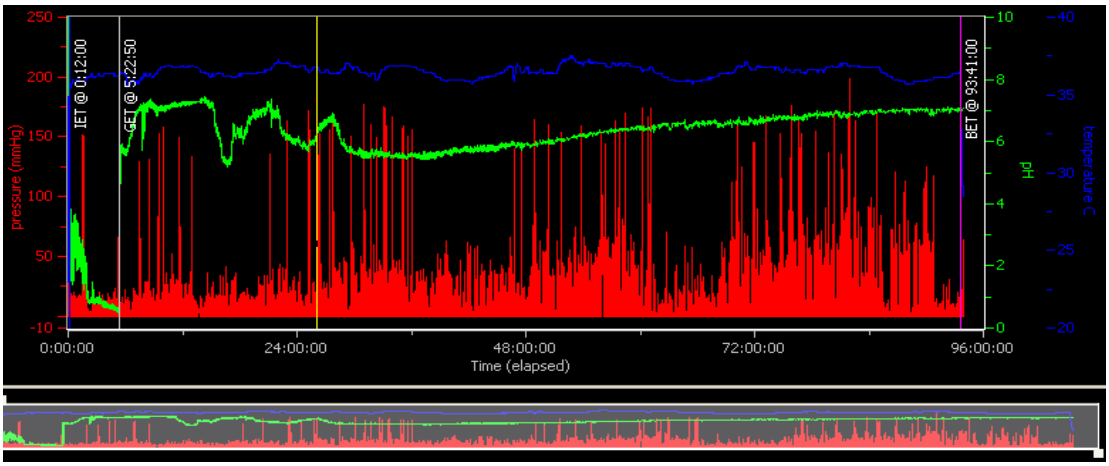


Figure 7.03. Examples of plot data obtained from WMC recordings from three STC patients. These show a marked increase in overall colonic contractile activity (shown in red) compared to a healthy control (Figure 7.01).

7.5.6. PROXIMAL COLONIC PRESSURE PROFILE

Proximal colonic contraction frequency (PCCF) and proximal colonic motility index (PCMI) in healthy volunteers and STC patients are presented in Table 7.04. No differences were found between groups.

	Group	N	Mean	SD	Min	Max	*P* value
PCCF	normal	50	3.2	2.5	0.1	11.3	0.5
	STC	18	3.2	2.3	0.1	10.0	
PCMI	normal	50	111.0	104.0	1.0	509	0.9
	STC	18	116.0	96.2	18.0	358	

Table 7.04. Proximal colonic pressure profiles in healthy volunteers and in STC patients during 60 minutes after following ICJ passage. PCCF: proximal colonic contraction frequency; PCMI: overall proximal colonic motility index; N: number; SD: standard deviation.

7.5.7. DISTAL COLONIC PRESSURE PROFILE

Distal colonic pressure profile measurements in healthy volunteers and STC patients are presented in Table 7.05. There were no differences in contractility parameters between groups.

	STC	N	Mean	SD	Min	Max	*P* value
DCCF	normal	53	3.0	1.5	0.7	10.0	0.9
	STC	16	2.3	1.3	0.3	4.6	
DCMI	normal	53	281.0	206.0	20.0	927.0	0.5
	STC	16	365.3	234.0	29.0	890.0	

Table 7.05. Distal colonic region pressure profiles in healthy volunteers and in STC patients during 60 minutes following ICJ passage. DCCF: distal colonic pressure frequency; DCMI: Overall distal colonic motility index; N: number; SD: standard deviation.

7.5.8. pH AROUND THE ICJ

In healthy volunteers, meal protocol was statistically significantly associated with a difference in pH around the ICJ (smaller magnitude of change for delta ICJ with protocol 2: *P* = 0.005) (Table 7.06).

Compared with healthy volunteers who followed meal protocol 2, STC patients showed statistically significant differences in delta pH values compared to healthy volunteers [Delta ICJ pH: median 1.2 (range: 0.1 - 2.8) in healthy controls vs. median 2.1 (range: 0.6 - 2.5) in STC patients; *P* = <0.005].

Parameter	Meal protocol	Gender	N	Mean	SD	Median	5th percentile	95th percentile
Delta ICJ in healthy volunteers	All	All	186	1.2	0.5	1.2	0.3	2.1
	1	F	28	1.4	0.4	1.3	0.8	2.1
		M	42	1.5	0.5	1.5	0.7	2.2
	2	F	50	1.2	0.5	1.2	0.4	2.0
		M	51	1.1	0.6	1.1	0.2	1.9
Delta ICJ in STC patients	2	All	18	1.8	0.6	2.1	0.6	2.5

Table 7.06. **Delta ICJ pH values in healthy volunteers and in STC patients.** N: number; SD: standard deviation.

7.6. DISCUSSION

To date, this is the largest reported data set that explores transit times and pressure profiles throughout the whole colon in healthy humans. Results were then compared to those obtained in a pilot sample of STC patients. The current study presents robust evidence that testing protocol and gender both influence CTT, which should therefore be taken into consideration when interpreting data in a clinical context. However, as a broad benchmark, the data presented herein demonstrates that if the WMC is not expelled by the 3rd morning after ingestion (i.e. 72 hours), transit through the whole gut (and at least 1 region of the GI tract including the colon) is pathologically delayed. For the colon, a residence time of 51 hours was shown to be the upper limit of normal, and hence can be used to define STC.

CTT was longer in females, mirroring previous observations (Metcalf et al., 1987, McLean et al., 1992, Bennink et al., 1999, Malagelada et al., 1984, Rao et al., 2009). For instance, Sadik *et al*. demonstrated in a study of 83 healthy controls, using a combined technique of ROM and fluoroscopy, that colonic transit was significantly slower in females (Sadik et al., 2003). This has been reflect hormonal differences between genders and proposed to that transit may vary according menstrual phase status although results from various studies are inconsistent (Chapter 1, section 1.6.1.3.4.1). For example, Wald *et al.* reported that GI transit time was prolonged in the luteal phase of the menstrual cycle in comparison to the follicular phase, thereby implying an effect of rising progesterone on retarding transit (Wald et al., 1981). However, other studies show no change in transit times between luteal and follicular phases (Hinds et al., 1989). The data collected in the current study did not include consideration of menstrual cycle and status.

The current study has also shown that age does not affect CTT. This is in agreement with the majority of previous studies that shown no correlation between age and increasing transit times (Metcalf et al., 1987, Merkel et al., 1993, Meier et al., 1995).

Nevertheless, CTT *was* significantly prolonged in subjects who followed meal protocol 2. This is similar for other regional transit times (gastric, small bowel) and whole gut transit; however, such data were omitted for brevity. As the WMC is an indigestible solid, its expulsion from the stomach is facilitated by distally propagating high amplitude antral contractions from phase III of the migrating motor complex (Minami and McCallum, 1984). This pattern occurs in the fasting state, so expulsion of WMC from the stomach is dependent on cessation of "fed state" stomach contractions, associated with the initial test meal (Cassilly et al., 2008), which clearly explains the effect of study protocol on gastric emptying time (GET). However, the reason behind prolonged CTT associated with meal protocol 2 is not obvious. As with regional transit times, testing protocol significantly influenced pH values in the ICJ (and also other GI regions; data again omitted as they are beyond the purpose of this research), supporting the need for a standardised protocol to be adopted.

One striking finding of this study was that in health, WGTT (and CTT) showed an interesting clustering of data values separated by 24 hours (Figure 7.02). These frequency peaks appeared to be the result of capsule expulsion with the first bowel movement of the day. It is known that both morning waking and meal consumption result in an increase in colonic contractile activity (Bampton et al., 2001), with the combined effect of both of these physiological stimuli thereby producing strong colonic contractions that precede defaecation; accordingly, CE is most likely to occur in this period. This finding is of major importance with regard to the performance of current ROM techniques. Given that the data presented in this study show that colonic (and also whole gut) transit cannot be described as a continuous variable (as promoted by several existing methods) (Metcalf et al., 1987, Abrahamsson et al., 1988), it could be proposed that a more physiological way of reporting whole gut (and colonic) transit time(s) is in increments of 24 hours. Our data demonstrated that 36% of subjects expelled the capsule by 24 hours, 85% by 48 hours, and 96% by 72 hours. Such an approach would, however, require that all subjects commence the investigation at the same time of the day, which is now the accepted protocol. Lack of standardisation remains a major limitation with almost all other contemporary tests of GI function, especially those involving radiology, where scheduling conflicts

present a logistical challenge to establishing a common ingestion time. The lack of use of standardised meals and scan times also continues to be problematic. In contrast to other GI motility testing, with the exception of high-resolution oesophageal manometry (Bredenoord et al., 2012), the WMC offers uniformity of test administration and interpretation.

In terms of comparing manual and automated analysis, subtle pH changes across the ICJ were poorly identified by the automated software analysis. While this also affected CTT, the longer time period of CTT meant that the difference was of a much smaller magnitude. Therefore, manual identification of ICJ should always be performed.

Another major finding of this study is that the overall colonic motility index was significantly *higher* in STC patients than in healthy controls, which may be counterintuitive ('classic' teaching suggests a paucity of propagating contractions in STC) (Figure 7.03) though not specifically in the proximal or distal colon. This probably reflects an increase in non-propagating retrograde colonic contractile activities, and the lack of suppression of antegrade activities during the nightime, that have previously shown in pancolonic manometric recordings (See Chapter 4). However, it is not possible to identify pressure wave propagation and polarity using the WMC, which is one of its limitations. The finding that proximal and distal colonic motility parameters were similar between groups, is inconsistent with previous findings obtained from pancolonic manometry that showed significant increases in antegrade and retrograde propagating contractile activities within the proximal colon of STC patients (Chapter 4). This is probably due to our inability to accurately localise the WMC during colonic passage. Only one other study has attempted to characterise the colonic pressure profile in constipated patients using the WMC (Hasler et al., 2009). This study showed no difference in contraction frequency between groups, consistent with the findings of this study. In addition, the Hasler *et al* study highlighted that there was a loss of the progressive increase in colonic contractile activities over colonic regions (from proximal to distal colon) in constipated patients, compared to healthy controls. However, the major limitation of

this study was that the authors subdivided the colon into four regions based on overall time spent in the colon. This is not justifiable, given that localisation of the WMC within the colon cannot be accurately determined. Even the method of equating the first hour after ICJ passage to represent the proximal colon and the last hour before CE to represent the distal colon is speculative, not fact. Further studies of colonic motility using the WMC perhaps in conjunction with imaging techniques (see Chapter 6) to confirm capsule position are required to establish regional differences of colonic pressure.

In patients with STC, this study showed that delta ICJ pH was significantly higher in this group compared to healthy volunteers, allied to lower pH values in the caecum. Such abnormalities in intraluminal GI pH may feasibly represent alterations in gut microbiota (dysbiosis) resulting in excessive production of short-chain fatty acids, which are associated with functional gut disorders including chronic constipation, and also with small bowel bacterial overgrowth (Simren et al., 2012).

The advantages and limitations of using the WMC technology over manometry in assessing gastrointestinal motility are summarised in table 7.07. One of the disadvantages of using the WMC is that radiographic imaging must be used to identify capsule retention when it fails to pass spontaneously. As with any other clinical test, the device can suffer signal loss and fail to record, which is reported to occur in around 0.8% of studies, as assessed in post-marketing analysis of the device (Saad and Hasler, 2011). In addition, prolonged CTT measured by WMC does not differentiate those who suffer from ‘primary’ slow transit from those with delayed transit secondary to defaecatory dysfunction, or indeed both. However, further studies exploring differences of colonic pressure profile among subgroups of constipation, may provide more insights to the underlying pathophysiology. A further limitation is that the WMC can only evaluate GI contractile activities at a single point, as opposed to manometric techniques where multiple recording points can record contractions simultaneously over a large area of the bowel, and therefore can detect propagating pressure contractions. However, due to major challenges associated with test performance, as well as the invasiveness of the colonic manometric

technique, along with a lack of normative data in addition to other limitations mentioned previously (Chapter 1, section 1.6.1.3.4), means the WMC may provide a viable alternative, or perhaps a complementary test for the clinical assessment of colonic function.

Measurements and techniques	Wireless Motility Capsule	Colonic manometry
Pressure recording	+	++
pH recording	++	-
Transit time	++	-
Multiple recording points	-	++
Polarity and propagation of pressure wave	-	++
Non-invasiveness	++	+
Simultaneous assessment of other regions of GI tract	++	-

Table 7.07. Comparison of measurements and techniques of colonic manometry and the wireless motility capsule.

One of the specific limitations of this study is that data were derived from several research centres. However, this is acceptable when all research centres follow well-defined inclusion and exclusion criteria and study protocol. Indeed, multicentre studies of novel interventions that may include physiological assessment, are positively encouraged to include multiple centres from several countries (Emmanuel et al., 2014). Furthermore, a proportion of STC patients did not have prior ROM studies to confirm delayed colonic transit nor lower GI physiological assessment to rule out the presence of other underlying pathophysiologies. However, all patients did have confirmed delayed colonic transit based on their WMC studies. Moreover, given that the WMC is not widely available in the UK and is not part of routine GI physiological workup, therefore we had to use collective data from other centres. The findings of the current study will form a platform for future prospective studies that ideally will involve more homogenous subjects. In addition, the determination of colonic motor activities and pH profile as measured by the WMC in other subgroups of constipated patients, warrants further investigation.

In conclusion, the WMC is an ambulatory, minimally invasive, non-radiological method for simultaneously determining gut transit times (including CTT), intraluminal pH, and also intraluminal pressure profile. This study demonstrated that in healthy subjects, colonic transit time is not a continuous variable and exhibited peaks (every 24 h), which could be used to redefine cut-off for normal colonic transit values. Furthermore, colonic transit times appear to be influenced by gender and testing protocol meaning results from an individual patient should be compared to appropriately stratified normative data sets. The study has also shown that in STC patients, pH in the caecum is significantly lower than in healthy controls, with an increase in overall colonic motility index compared to healthy subjects. The development of an internationally accepted protocol remains the crucial next step for cross-referencing of data in both the clinical setting and for research purposes.

8 SUMMARY, KEY FINDINGS, CONCLUSIONS, AND FUTURE STUDIES

8.1. THESIS OVERVIEW AND GENERAL RESEARCH AIMS

Constipation is one of the most common gastrointestinal symptoms volunteered by adults and paediatric population. Slow transit constipation (STC) represents a subgroup of constipated patients who usually present with severe refractory symptoms. STC is a measurement-based physiological disorder and is based on delayed colonic transit as defined by transit studies (radio-opaque markers or isotope scintigraphic studies). The pathophysiology of STC is poorly understood; however, dysregulation of colonic motility is considered a crucial aetiological hypothesis. Direct assessment of colonic contractile activities throughout the colon can be measured using the technique of colonic manometry. However, this technique is invasive, not standardised and technically challenging; consequently our understanding of pan-colonic motility in health and in disease (including STC) remains rudimentary. Previous studies of colonic contractile activity in STC have shown some variability in findings that are likely (in part) attributable to the use of various recording techniques and study protocols. Moreover, most of these studies failed to report colonic motor activities from proximal colonic regions and are the majority limited to the distal colon. Generally, patients with STC are reported to have a reduction in the number, amplitude and duration of colonic high amplitude propagating sequences (HAPS). An absent or attenuated colonic motor response to physiological stimuli such as meal or to stimulant laxatives such as bisacodyl is also reported in STC. Detailed assessment of pan-colonic contractile activities using a more standardised technique is fundamental to enhancing our understanding of colonic motility, which could potentially better direct medical and surgical intervention for patients with colonic dysmotility (i.e. STC). However, the utility of colonic manometry as a clinical tool for adult populations is still poorly recognised, unlike for paediatric patients. With recent advances in colonic manometric recording techniques and the availability of long manometric catheters that span the whole length of the colon, the studies performed within this thesis primarily aim to provide a more detailed understanding of colonic propulsive motor activities in the healthy human colon in basal physiological conditions, and to attempt to better characterise

such activities in STC patients. Other research aims are: (1) to determine the effect of recording methodologies on pancolonic motor activities; (2) to validate the use of a new indigestible capsule technology as a minimally invasive tool to measure colonic motility in both health and disease (specifically STC).

8.2. PANCOLONIC MOTOR FUNCTION IN HEALTH: INFLUENCE OF BOWEL PREPARATION

8.2.1. SUMMARY

Lack of standardisation of the manometric technique used to assess pancolonic motor function is considered a principal limitation. Colonic manometric catheters can be introduced by a retrograde (per-rectal) or antegrade (per-nasal) approach. In order to determine the impact (if any) of prior bowel preparation on colonic motor activities in the healthy human colon (specifically, the characteristics of propagating sequences (PS), spatiotemporal organisation, colonic meal response, and stereotypic predefaecatory motor patterns), the two techniques were compared. Eight subjects underwent water-perfused pancolonic manometry, using per-rectal colonic intubation with prior bowel preparation. A further group of 8 healthy subjects underwent per-nasal colonic intubation without prior bowel preparation. Although the two study groups were investigated in separate research centres, all healthy subjects followed similar inclusion criteria, an identical study protocol and similar assembly of recording catheters.

The results of the studies showed that prior bowel preparation can influence some parameters of colonic motor function, namely: (i) characteristics of HAPS; (ii) the frequency and amplitude of PS; (iii) the relationship between consecutive PS, such as ‘linkage’; and (iv) stereotypical pre-defaecatory patterns. However, colonic motor responses to commonly assessed physiological stimuli such as meals and morning waking were similar between groups.

8.2.2. CONCLUSIONS

The retrograde intubation of colonic manometric catheters into a prepared colon is technically more achievable than pernasal intubation, but has always been criticised for adding an additional study confounder (i.e. the need of bowel preparation). The results of the current study show that, within limits, investigators can make a valid comparison between studies of pancolonic motility, with or without prior bowel preparation, when evaluating overall PS characteristics, the colonic meal response, nocturnal suppression of PS, and morning waking. Adoption of a retrograde intubation approach can thus be applied to a wider range of research studies in both healthy subjects and patients suffering from STC, where the colon is usually loaded with faeces and the use of bowel cleansing is essential. A further benefit is that, prior bowel preparation reduces the time required to achieve pancolonic intubation and provides a more practical and acceptable technique for patients.

8.3. PANCOLONIC SPATIOTEMPORAL MAPPING REVEALS DISORGANISATION OF COLONIC PROPAGATING PRESSURE WAVES IN SLOW TRANSIT CONSTIPATION

8.3.1. SUMMARY

Few manometric studies have been published describing pancolonic motor activities in STC. The majority have recorded motility from sites distal to the mid-transverse colon, many confined to the descending or sigmoid colon only. In this study, pancolonic manometry (using water-perfused catheters introduced per-rectally following prior bowel preparation) was performed in 14 patients with STC (as previously confirmed by colonic scintigraphy) and in 8 healthy volunteers. Detailed, colour-contoured spatiotemporal maps of PS activity from the caecum to the anorectum were constructed to provide a better appreciation of colonic motor activities. For the first time, potentially important new phenomena have been

revealed in patients with STC, specifically: (i) a relatively adynamic region around the splenic flexure, which appears somewhat ‘disconnected’ from the adjacent proximal and distal colon, and in which there is a marked paucity of propagating pressure waves; (ii) a marked reduction in the extent of propagation of antegrade PS in the proximal colon; (iii) poor regional linkage among consecutive PS throughout the colon; (iv) an increase in the frequency of proximal colonic retrograde PS; and (v) absence of the normal nocturnal suppression of antegrade PS. The study also confirmed findings of an absent meal response (gastrocolonic response) in most patients, and reduced frequency and amplitude of HAPS in patients with STC.

8.3.2. CONCLUSIONS

The results of this study demonstrate a striking disorganisation of overall spatiotemporal patterning among consecutive colonic PS in STC. This helps to explain the delay in colonic transit in patients with STC, which appears secondary to *dysregulated* colonic motor function, rather than to the traditionally accepted view of a reduction in overall colonic motility. Whether this pattern is a reflection of underlying cause (i.e. neuropathy and/ or myopathy) is still unknown and merits further research. This appears to predominate within the proximal colon. Nevertheless, such findings can potentially serve as manometric ‘signatures’ in patients with STC. However, other subgroups of patients with constipation should be included in broader studies of colonic motility in constipation. Such studies may lead to the identification of novel biomarkers that may serve as therapeutic targets.

8.4. MANOMETRIC ASSESSMENT OF PANCOLONIC MOTOR FUNCTION: COMPARISON BETWEEN SOLID-STATE AND WATER-PERFUSED TECHNOLOGIES

8.4.1. SUMMARY

To date, the vast majority of pancolonic manometric studies in adults have utilised water-perfused catheters introduced per-rectally with colonoscopic assistance. However, the need for continuous water perfusion during the study period (which often exceeds 24 hours) and non-ambulation of the subject under study, thus restricting them to the laboratory, are fundamental limiting factors. Solid-state catheter studies have thus far been mainly performed in the distal colon only.

In this study, we aimed to investigate pancolonic motor activities under more physiological conditions (i.e. by eliminating the effect of water perfusion), with the use of a custom-built solid-state catheter. Studies were compared with recordings obtained from water-perfused catheters (similar in design and specification to the solid-state catheter) within the same subject, but performed on different occasions. Six healthy subjects completed both manometric studies.

In contrast to earlier reports, describing the colon as a “relatively inactive” organ, near continuous motor activity was evident throughout the colon over the entire recording period when studied using solid-state technology. There was a significant increase in the frequency of overall PS in both directions (i.e. antegrade and retrograde), and qualitative and quantitative analyses of solid-state studies additionally showed a striking loss of suppression of PS during the nocturnal period, as well as a loss of the waking response in comparison to water-perfused studies. Such observed changes are likely due to better fidelity of the solid-state catheter system, as confirmed by allied bench studies. Significant regional colonic differences recorded by water-perfused technology manifested as longer propagation distance of antegrade PS in the right colon, and higher amplitude of retrograde PS in the distal colon; this may be attributed to a difference in consistency of colonic content within the colon.

Augmentation of PS following a meal (i.e. the gastrocolonic response) and HAPS characteristics were similar in both groups.

8.4.2. CONCLUSIONS

'Solid-state' technology has been available for a few decades to measure colonic motor activities however; it had never been used previously to record 24-hour pancolonic motor activities from multiple recording sites. For the first time, this has been achieved using a custom-made solid-state pancolonic manometric catheter. Results from these studies showed a loss of the normal nocturnal suppression of colonic PS in solid-state catheter recordings, which has been accepted as a key element of the normal circadian rhythm. This finding highlights the inherent differences in reported colonic motor activities that are clearly secondary to technological variability. Other parameters that define individual PS (including amplitude, frequency, and propagation) are also influenced by recording techniques, and hence this should always be taken into consideration when interpreting studies in which different recording technologies have been used. Nevertheless, other important key features of colonic motility (e.g. the colonic meal response and HAPS activities) appear to be equivalent, irrespective of recording methods. These parameters can therefore be compared in a valid way between data derived from different recording methods. This is important as both factors have been shown to be altered in colonic motility disorders (including slow transit constipation), both in adult and paediatric populations. HAPS activities associated with the process of defaecation, thus future studies ideally requiring a minimum of 24 h recording, to include assessment of these activities. Nevertheless, the use of provocation test (eg. instillation of bisacodyl to elicit HAPS) may prove more practical in shorter studies. The use of 'solid-state' technology potentially offers a more practical recording tool for colonic manometry recordings in both research and the clinical setting.

8.5. ACCURATE ASSESSMENT OF COLONIC TRANSIT TIMES USING THE WIRELESS MOTILITY CAPSULE: A VALIDATION STUDY TO LOCATE THE FALL IN PH WITHIN THE ILEOCAECAL REGION USING A DUAL-SCINTIGRAPHIC TECHNIQUE

8.5.1. SUMMARY

Although colonic manometry is considered the 'gold standard' for the direct measurement of colonic motor function, the difficulty in performing such studies has limited the use of this technique to only a few research centres worldwide. As an indirect measure of colonic motor function, it is now accepted that measurement of colonic transit time (CTT) should be the initial test of choice for assessing colonic dysmotility. This can be achieved in clinical practice by two radiological techniques: radioopaque markers and colonic scintigraphy. These methods, however, have limitations as both involve irradiation and there is a lack of standardisation. Furthermore, a proportion of patients with STC may present with symptoms of a panenteric motor disorder and only whole gut scintigraphy, which is extremely time-consuming, can detect transit abnormalities in distinct gut regions. Alternatively, GI transit times can be derived from stereotypical changes in pH profile as recorded from within the lumen of the gut. This can be assessed through the use of ingestible telemetric capsules. A fall in pH around the ileocaecal junction (ICJ) has been proposed as a landmark for colonic entry. However, the validity of this pH change has never been appropriately investigated.

In this study, we aimed to determine the anatomical site of the fall in pH around the ICJ, using a dual-scintigraphic technique, and thus confirming whether this pH change can truly be used as a precise biomarker of transition from small to large bowel. Thirteen healthy volunteers were enrolled for this study. On day 1, they underwent nasal intubation with a 3 m long catheter, which was allowed to progress to the distal ileum. On day 2, subjects ingested a pH-sensitive wireless motility capsule (WMC) labelled with ^{51}Chromium [EDTA]. Position of the WMC, as it

travelled through the GI tract, was assessed with a single-headed-gamma camera using static and dynamic scans. Capsule progression was plotted relative to a background of ^{111}Indium [DPTA] administered through the catheter. Intraluminal pH, as recorded by the capsule, was monitored continuously, and the position of the capsule in relation to pH change was established.

The study showed a sharp fall in pH in all subjects; the position of the capsule relative to this pH drop was accurately determined anatomically in nine subjects. This occurred either in the caecum (5 subjects), ascending colon (2 subjects), or as the capsule moved from the caecum to the ascending colon (in 2 subjects). Overall, the magnitude of the pH drop was 1.45 ± 0.20, to a nadir pH value of 6.1 ± 0.1. The onset of fall in pH occurred at a median of 7.5 min after passage through the ICJ. All subjects expelled the capsule within 30 h after oral ingestion (median whole gut transit time was 20.3 h) with no adverse events being recorded. Median colonic transit time was 11.9 h as measured by WMC.

8.5.2. CONCLUSIONS

The study confirmed the previously described characteristic fall in pH around the ileocaecal region and showed that the fall actually occurs in the proximal colon. This phenomenon can thus be used as a biomarker of transition between the small and large bowel, and validates the assessment of regional GI motility using WMC technology that incorporates pH, pressure and temperature measurements. The WMC can potentially be used in subsets of patients as part of their clinical workup, to provide a more detailed assessment of whole gut transit in addition to pH profile along the gut. This technology can hence also be used as a minimally invasive test to monitor the response to various medical and surgical interventions as the investigation does not involve radiation exposure and can be easily repeated. However, the cost of the WMC is expensive compared to other ‘reference standard’ tests such as radio-opaque markers.

8.6. STUDIES OF COLONIC MOTILITY (TRANSIT TIMES AND CONTRACTILE ACTIVITIES) USING THE WIRELESS MOTILITY CAPSULE: COMPARISON BETWEEN HEALTH AND SLOW TRANSIT CONSTIPATION

8.6.1. SUMMARY

The WMC offers measurement of GI transit times and contractile activities simultaneously, in a minimally invasive manner. To further validate assessment of colonic transit time (CTT), we sought to investigate the effect of gender, age and testing protocol in a large cohort of healthy volunteers. For assessment of colonic contractility, this is based on anatomical landmarks, and as such can only be realistically evaluated in the right colon (caecum and ascending colon, based on knowledge of ICJ passage), and distal colon (based on knowledge of subsequent capsule expulsion). From a large cohort of healthy subjects, we primarily aimed to provide normative data for colonic contractile events, and a further pilot study was then performed to compare these measures with results derived from a small group of patients with STC.

Regional GI transit (including CTT) and pH values were determined in 215 healthy volunteers. Proximal and distal colonic pressure profile (contractility) data were obtained from a subset of 54 healthy subjects, and all measures were compared with those obtained from 19 patients with STC.

The main findings of this study were: (i) the upper limit of normal CTT was 51 h; (ii) CTT is significantly prolonged in females; (iii) CTT can be significantly influenced by study protocol; (iv) CTT appears to occur as frequency peaks separated by 24 h. With regard to colonic contractility and pH profile within the colon: (i) overall colonic motility index was significantly higher in STC patients than in healthy controls, with no apparent regional difference; (ii) delta ICJ (the difference between median ileal pH value and median caecal pH value around the time of WMC passage through the

ICJ) was significantly greater in patients with STC, allied to lower pH values in the caecum.

8.6.2. CONCLUSIONS

1. Measurement of CTT using the WMC varies based on gender and testing protocol. Therefore, results of CTT derived from the WMC should be stratified by sex, and should only be compared with normative data derived using a similar study protocol. A standardised testing protocol can be easily adopted using this technology and ideally should be applied in all centres. This will facilitate worldwide data sharing and study comparison among centres;

2. The study showed that WGTT and CTT exhibits peaks every 24 h, consistent with human bowel habit. This brings into question the concept of transit time as a continuous variable, as utilised by radio-opaque marker studies. It is thus proposed that clinical measurement of CTT should be performed in increments of 24 hours with upper limit of normal being 3 days;

3. Colonic contractility as measured by the WMC appears to be increased in STC compared to healthy subjects. This is consistent with the findings from pancolonic manometry studies, supporting the concept that colonic motility is dysregulated rather than impaired in STC. However, the WMC is unable to provide information on the polarity and propagation of colonic motor activities as it only measure pressure changes based at a single recording point. Thus the ‘gold standard’ test for measuring colonic motility remains as colonic manometry. Nevertheless, the United States Food and Drug Administration (FDA), have approved the WMC for the measurement of GET in patients in whom gastroparesis is suspected, the evaluation of CTT in patients with suspected slow transit constipation, and the measurement of pH, pressure and temperature throughout the GI tract. The American and the European Neurogastroenterology and Motility Societies have endorsed these indications in a recently published position paper (Rao et al., 2011).

4. Delta ICJ is significantly greater in STC compared to healthy volunteers. Alteration in gut microbiota (dysbiosis), resulting in the excessive production of short-chain fatty

acids, is proposed as an underlying pathophysiological mechanism. A recent paper by Farmer *et al.* has also reported differences in both caecal pH and delta ICJ pH in patients suffering from 'irritable bowel syndrome' compared to healthy controls (Farmer et al., 2014). The authors concluded that these measures, as recorded by the WMC, could be used as surrogate biomarkers of fermentation, potentially identifying those patients that may preferentially benefit from antibiotic or dietary interventions.

8.7. CONCLUDING REMARKS

Our knowledge of normal (and hence abnormal) human colonic motor function (motility), and its governing mechanisms, remains incomplete. Historically, this has been due to the relative inaccessibility of this organ for study, as well as a lack of standardisation of methods used to investigate it. Nevertheless, recent device development has provided us with advanced tools, namely pancolonic manometry, and also an ingestible, pressure-sensing, telemetric capsule (the wireless motility capsule), by which to assess colonic motility. The clinical area for the use of such (potentially diagnostic) tests is functional bowel disorders, with constipation being the second most commonly self-reported gastrointestinal symptom. A sub-group of patients presenting with severe intractable symptoms, but without organic disease, are found using traditional diagnostic tests to have slow transit constipation (STC), which is believed to be due to colonic dysmotility.

The studies performed within this thesis have revealed that in healthy subjects, pancolonic manometric recording technique (including prior bowel preparation and catheter type), significantly influences the characteristics of colonic motor function (specifically propagating pressure waves). From both a research and clinical perspective, this is very important, as investigators need to take such variation into consideration when comparing data derived from different techniques.

In patients presenting with slow transit constipation, we have shown, for the first time, that motor activities appear to be dysregulated throughout the colon, rather than simply suppressed as previously thought, along with loss in ‘regional linkage’ of pressure waves.

This thesis also describes a novel technology (the wireless motility capsule) for measuring colonic motility. An initial validation study showed the device was able to detect a significant pH fall within the proximal colon, which can be used as an indicator for capsule entry into the large bowel. Therefore, colonic transit time can be accurately measured.

Study of a large cohort of healthy subjects using the WMC showed that colonic transit time is not a continuous variable. This finding is of major importance as it emphasises the urgent need to review the performance of current techniques (i.e. radio-opaque markers). Our studies indicate that colonic transit time should be described in increments of 24 hours.

Finally, a pilot study in patients with slow transit constipation showed an overall increase in colonic motility as measured by this device. This is in agreement with our findings from pancolonic manometric studies. Taken together, these observations have revised our understanding of the pathophysiology of STC.

8.8. FUTURE STUDIES

The studies performed in this thesis have provided further insight into the physiology of colonic motor functions, and also pathophysiology in patients with slow transit constipation. As such, these results form a platform for further investigation in this field. The most important question that remains is the clinical utility of colonic motility studies to define subgroups of patients based on manometric ‘signatures’ or biomarkers. This would likely aid in the development of a better management strategy and perhaps offer new therapies targeted toward dysregulated colonic motility.

Nevertheless, further research is required to further evaluate the tools for assessing colonic motility:

8.8.1. COLONIC MANOMETRIC STUDIES

What is the impact of using more advanced technology in recording pancolonic motor activities on the quantitative and qualitative data?

Recently, high-resolution fibre-optic manometric catheters, incorporating up to 120 sensors spaced at 1 cm intervals have become available (Dinning et al., 2013, Dinning et al., 2014). On the basis of the available literature and our findings in chapter 3 and 5, there is now general agreement that recording technology and the specification of the recording catheter (including numbers and spacing of recording channels) will influence qualitative and quantitative result of manometric recordings. 'High-resolution' manometric technologies with new colour-contoured topographical plots have lead to advances in understanding of oesophageal and anorectal diseases. These techniques have been widely adopted with proven clinical utility. The use of similar technology to record colonic motility may open a new window for colonic manometry (realistically performed on the left-side only, given the difficulties associated with pan-colonic intubation) to move forward as a clinical tool. However, this must be formally tested;

Using the above technology, a well-designed and adequately powered study involving constipated patients is required to determine whether certain manometric biomarkers are able to subclassify patients into more homogenous subgroups

Studies performed in chapter 4 revealed the presence of significant qualitative and quantitative difference in colonic motor activities recorded in STC patients versus those obtained in healthy subjects. However, some of these findings have been found to be present in other subgroups of constipation such as obstructed

defaecation. Hence an adequately powered study, incorporating all known subgroups of constipated patients is warranted to determine if certain 'manometric signatures' can define patient (sub) population and direct better management. Use of high-resolution catheters may provide further (as yet unrecognised) information (either diagnostic or prognostic).

What is the effect of performing colonic manometric studies in short colonic segments (i.e. distal colonic manometry) versus pancolonic manometry?

All available literatures and results obtained from studies in chapters 3, 4, and 5 have highlighted the difficulties in performing pancolonic manometric studies in a wider context due to its inherent invasiveness. Therefore, such studies remain limited mainly to research and in a very few clinical centres worldwide. To move forward, it is essential to investigate whether studying distal colonic motor function provides sufficient pathophysiological information in constipated patients to obviate the need for a full pancolonic assessment.

What's the effect of adjusting study protocol adopted for pancolonic manometric studies?

The long duration of pancolonic manometric studies (usually 48 h) is required to remove the effect of prior bowel preparation and recording circadian rhythm. Studies performed in chapter 5 showed that the use of specific recording technology affect the characteristic circadian rhythm of colonic motor activities (i.e. loss of nocturnal suppression). Conversely, other results from chapter 5, studies in chapter 4, and also several previous studies, confirm that studying the meal response and HAPS characteristics are important and consistently exhibited colonic manometric parameters that may be significantly different between health and disease. Therefore, it is essential to investigate whether adopting a shorter study protocol (for $\approx$ 4 hours, to include fasting and post-meal recording), is sufficient to provide detailed pathophysiological information in constipated patients that would eliminate the need for more prolonged studies. Short duration pancolonic manometric studies have

been clinically adopted in paediatric patients, and the results of these studies have differentiated patients into subgroups, based on their underlying pathophysiology. Accordingly, these results are used to guide medical and surgical management. Finally, a provocation test (for example intraluminal administration of bisacodyl) could be routinely adopted at the end of shorter pancolonic studies. Again, whether a short vs. longer protocol provide sufficient pathophysiological information must be tested.

8.8.2. WIRELESS MOTILITY CAPSULE STUDIES

To develop an internationally accepted study protocol for performing WMC studies

This would allow test standardisation and enable cross-referencing of data between study centres in both the clinical setting and for research purposes. Studies performed in chapter 7 showed that study protocol can significantly influence various data obtained from the WMC. The technology of the WMC can easily be adopted into a standardised protocol, unlike other methods used to determine GI transit such as radio-opaque markers. Although cost-effectiveness is a limitation, establishing a universally accepted protocol in such a minimally invasive test may place it at the top of the list of clinical tools used to assess whole gut and regional GI transit.

What is the difference (if any) in the recorded colonic pressure activities in constipated patients obtained from WMC recordings?

The pilot study described in chapter 7 showed a significant increase in the motility index in STC compared to healthy subjects. A wider study involving a larger cohort of constipated patients (including those with normal and slow colonic transit) is needed to investigate the clinical utility of this device in subclassifying patients based on patterns of colonic contractility. All patients should also have a detailed assessment of their rectal evacuation and to investigate whether the presence of rectal

evacuatory dysfunction can affect the patterns of colonic contractility as determined by the WMC.

What is the difference (if any) in the recorded pH profile around the ICJ and throughout the colon in constipated patients?

The pilot study described in chapter 7 showed a significant difference in pH profile recorded around the ICJ in STC patients, compared to healthy subjects. Whether this is a characteristic finding in STC is unknown. As above, further studies in a larger cohort of constipated patients are required to determine pH profile along the colon, establish its clinical utility, and to differentiate underlying pathophysiologies.

What is the ability of other indigestible wireless motility capsules to assess regional colonic motor functions?

The magnetic tracking system (Guignet et al., 2006, Hiroz et al., 2009, Hedsund et al., 2013) is now available for recording colonic motor activities. However, such technology needs further assessment to determine its ability to provides more detailed information than the existing WMC. Furthermore, endoluminal image analysis obtained from capsule endoscopy (PillCam SB video capsule, Given imaging) using specialised computer analysis software offered a reliable and minimally invasive method to assess small bowel motility (Malagelada et al., 2008). Again, whether similar technology (with a longer battery life) is able to assess colonic motility merits further investigation.

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APPENDICES

1. The standard bowel symptom questionnaire of the Lower GI Physiology Unit at the Royal London Hospital provided to patients prior to their appointment.

ANORECTAL DYSFUNCTION IMPACT SCORE

Name:……..

Date of Birth:……

Today's Date:……

This questionnaire will save time when you attend for your bowel tests, and is designed to gain important information about your symptoms and how much they affect you.

PLEASE BRING IT WITH YOU, AND HAND IT TO ONE OF THE GI PHYSIOLOGY UNIT STAFF WHEN YOU ARRIVE FOR YOUR TESTS.

INSTRUCTIONS

This questionnaire consists of 5 sections. Please complete all sections and answer every question by ticking the appropriate box. If you are unsure about how to answer a question, give the best answer you can. Some of the questions may look like others, but each one is different so please try to answer all of them. Some of the questions listed on the following pages will ask you how much each of your symptoms bother you. When answering these questions try to think how much each symptom is a problem for you at the moment in terms of how it affects your day to day life (eg. does it stop you doing things that are important to you?) and your general well being. You will then be asked to mark on a scale from 0 to 10, how much you feel each symptom bothers you (0 = not at all, 10 = severely).

To score please circle the appropriate number, for example:

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

If you answer that you NEVER suffer with that particular symptom then you do not need to score how much this symptom bothers you.

SECTION 1

1. Do you suffer with constipation? o Never o Yes

If Yes:

How long have you suffered with it?

o Less than 12 months

- o 12 months to 4 years
- o 5 to 9 years
- o 10 to 19 years
- o 20 years or more (or all of your life)

How much does constipation bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

2. How often do you open your bowels?

- o more than 5 times each day
- o approx 3-5 times each day
- o 1-2 times every 1-2 days
- o about 2 times each week
- o about once each week
- o about once every 10 days
- o less than once every 14 days

How much does this bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

3a. What is the usual consistency of your stools?

- o Watery, no solid pieces
- o Mushy, fluffy pieces with ragged edges
- o Soft blobs, with clear edges (passed easily)
- o Sausage-like, smooth surface (soft)
- o Sausage-like, but with cracks on the surface
- o Lumpy (may be sausage-shaped)
- o Hard lumps, like nuts / pellets (hard to pass)
- o Variable

3b. IF your stools are hard and / or “pellet-like”, how often does this occur?

- o Rarely (less than a quarter of the time)
- o Occasionally (a quarter to half of the time)
- o Usually (more than half of the time)
- o Always

How much does the hardness of your stools bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

4. On average, how long does it take to empty your bowels?

- o less than 5 minutes
- o 5 to 9 minutes
- o 10 to 19 minutes
- o 20 to 29 minutes
- o more than 30 minutes

How much does this bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

5. Do you take laxative medication by mouth (not enemas)?

o No o Yes

If Yes, how often is it effective?

o Never

o Rarely (less than a quarter of the time)

o Occasionally (a quarter to half of the time)

o Usually (more than half of the time)

o Always

6. Do you require any of the following assistance to pass motions?

(You may tick more than one box)

o I use enemas / suppositories

o I put my fingers in my vagina

o I put my fingers in my back passage

o Other, please describe _____

7. How often do you require such assistance to pass motions?

o Never

o Rarely (less than a quarter of the time)

o Occasionally (a quarter to half of the time)

o Usually (more than half of the time)

o Always

How much does this bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

8. How often do you need to strain when emptying your bowels?

o Never

o Rarely (less than a quarter of the time)

o Occasionally (a quarter to half of the time)

o Usually (more than half of the time)

o Always

How much does straining bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

9. How often when you try, are you unable to pass ANY motions?

o Never

o Rarely (less than a quarter of the time)

o Occasionally (a quarter to half of the time)

o Usually (more than half of the time)

o I always use my fingers to empty my bowels

How much does this bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

10. How often do you feel that you have not completely emptied your bowels following a bowel movement?

o Never

o Rarely (less than a quarter of the time)

o Occasionally (a quarter to half of the time)

o Usually (more than half of the time)

o Always

11. How much does this feeling bother you? Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

10. How often do you sense a 'blockage' that prevents you, or makes it difficult for you to open your bowels easily?

o Never

o Rarely (less than a quarter of the time)

o Occasionally (a quarter to half of the time)

o Usually (more than half of the time)

o Always

12. How much does this sensation bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

11. How often is passing motions painful?

o Never

o Rarely (less than a quarter of the time)

o Occasionally (a quarter to half of the time)

o Usually (more than half of the time)

o Always

13. Where do you feel this pain?

o Abdomen/tummy

o Back passage

o Vagina

o Other, please describe _____

How much does this pain on passing motions bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

14. Do you suffer with abdominal/tummy pain?

o Never

o Rarely (less than a quarter of the time)

o Occasionally (a quarter to half of the time)

o Usually (more than half of the time)

o Always

How much does abdominal pain bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

15. How often do you suffer with abdominal bloating that

leads to nausea or vomiting?

o Never

o Rarely (less than a quarter of the time)

o Occasionally (a quarter to half of the time)

o Usually (more than half of the time)

o Always

How much does the bloating bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

16. Do you pass blood from your back passage?

o No o Yes

17. Do you pass slime/mucus from your back passage?

o No o Yes

18. Do you associate the need to empty your bowels with

any of the following? (you may tick more than one box if applicable)

o A feeling/pressure in my back passage/rectum

o Cramping/pain in my abdomen/tummy

o Abdominal/tummy bloating

o None of the above, I go because I believe I should/out of routine

o Other, please describe_____

19. Do you remember having any problems with your bowels,

or going to the toilet as a child?

o No o Yes

If Yes, give details below

SECTION 2

1. How often are you incontinent to solid/formed stool?

o Never -> GO TO QUESTION 2

o Less than once a month

o Less than once a week but more than once a month

o Less than once a day but more than once a week

o Once per day or more

How long have you suffered with it?

o Less than 12 months

o 1 to 4 years

o 5 to 9 years

o 10 to 19 years

o 20 years or more (or all of your life)

How much do you lose?

o smear (pea-size)

o equivalent to half an egg cup full

o whole motion

Do you leak (you may tick more than one box):

o without being aware of it at first?

o when you have great urgency and cannot get to the toilet in time to open your bowels?

o when you cough, sneeze or run?

o following a bowel movement?

How much does this incontinence bother you? Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

2. How often are you incontinent to liquid/loose stool/slime?

o Never -> GO TO QUESTION 3

o Less than once a month

o Less than once a week but more than once a month

o Less than once a day but more than once a week

o Once per day or more

How long have you suffered with it?

o Less than 12 months

o 1 to 4 years

o 5 to 9 years

o 10 to 19 years

o 20 years or more (or all of your life)

How much do you lose?

o smear (pea-size)

o equivalent to half an egg cup full

o whole motion

Do you leak liquid/loose stool/slime (you may tick more than one box)

o without being aware of it at first?

o when you have great urgency and cannot get to the toilet in time to open your bowels?

o when you cough, sneeze or run?

o following a bowel movement?

How much does this incontinence bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

3. How often are you incontinent to wind?

o Never -> GO TO QUESTION 4

o Less than once a month

o Less than once a week but more than once a month

o Less than once a day but more than once a week

o Once per day or more

How long have you suffered with it?

o Less than 12 months

o 1 to 4 years

o 5 to 9 years

o 10 to 19 years

o 20 years or more (or all of your life)

How much does this incontinence bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

4. How often does your incontinence prevent you from doing everyday things (e.g. leaving the house, dressing, shopping, cleaning etc)?

o Not Applicable - I do not suffer with incontinence

o Never

o Less than once a month

o Less than once a week but more than once a month

o Less than once a day but more than once a week

o Once per day or more

5. Do you wear pads or anal plugs because of your incontinence?

o Not Applicable - I do not suffer with incontinence

o No

o Yes

How much does having to use these bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

6. Do you take Imodium, codeine or any other constipating

medications on a daily basis? o No o Yes

How much does having to use these bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

7. Can you “hold on” for 15 minutes when you feel

the need to open your bowels?

o No o Yes

If NOT, how long can you “hold on” for _____

How much does not being able to hold on bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

8. Are you ever incontinent of faeces because you mistake it for wind?

o No o Yes

How much does this bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

SECTION 3

1. Do you usually have a feeling of 'bulging' or something coming down (a 'lump') from the back passage? o No o Yes

If NO go to Section 4

How much does this bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

2. Can you see it? o No o Yes

3. When does it happen?

o unpredictable

o when I strain excessively

o following a bowel motion

o during exercise

o continuously

4. To make the 'bulge' / 'lump' go back, what do you have to do?

o Nothing, it goes back by itself

o Push it back with my finger

o I can't push it back myself

o Other, please describe _____

How much does this bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

5. Does mucus or blood ever come from the 'bulge' / 'lump'?

o No o Yes

SECTION 4

1. How would you describe your health at present?

o Very Good

o Good

o Fair

o Poor

o Very Poor

2. Overall, to what extent do your bowel symptoms interfere with your life?

o Not at all

o A little bit

o Moderately

o Quite a bit

o A lot

3. Please list the three bowel symptoms that bother you the most

1. _____

2. _____

3. _____

4. To what extent do your bowel symptoms affect your ability to perform daily tasks (e.g. dressing, shopping, cleaning etc)?

o Not at all

o A little bit

o Moderately

o Quite a bit

o A lot

5. To what extent do your bowel symptoms affect your ability to perform physical tasks (e.g. lifting, walking, running or sport etc)?

o Not at all

o A little bit

o Moderately

o Quite a bit

o A lot

6. To what extent do your bowel symptoms interfere with

your social activities (e.g. visiting friends, eating out, entertainment)?

o Not at all

o A little bit

o Moderately

o Quite a bit

o A lot

7. To what extent do your bowel symptoms interfere with your work?

o Not at all

o A little bit

o Moderately

o Quite a bit

o A lot

8. Approximately how many days have you needed to take off work, directly as a result of your bowel symptoms in the last year?

o Not applicable
o 0 - 4 days
o 5 - 9 days
o 10 - 14 days
o 15 - 19 days
o 20 days or more

9. To what extent do your bowel problems affect your relationship with your partner?

o Not applicable
o Not at all
o A little bit
o Moderately
o Quite a bit
o A lot

10. To what extent do your bowel problems affect your sex life?

o Not applicable
o Not at all
o A little bit
o Moderately
o Quite a bit
o A lot

11. Do your bowel problems make you feel depressed/feel bad about

yourself?

- o Not at all
- o A little bit
- o Moderately
- o Quite a bit
- o A lot

12. Do your bowel problems make you feel worn out/tired?

- o Not at all
- o A little bit
- o Moderately
- o Quite a bit
- o A lot

13. Do your bowel problems make you feel nervous or anxious?

- o Not at all
- o A little bit
- o Moderately
- o Quite a bit
- o A lot

SECTION 5

Do you suffer with any of the following?

Diabetes o No o Yes

Irritable Bowel Syndrome (IBS) o No o Yes

Crohns / Ulcerative Colitis o No o Yes

Lower back pain/ injury o No o Yes

Neurological conditions e.g. M.S. o No o Yes

Depression, anxiety, panic attacks o No o Yes

or other problems with your nerves

If Yes to any of the above please give details below

2. Do you suffer with any other medical conditions?

_____

3. Have you ever had an operation on your back passage

e.g. piles, fistula, tears (fissures) etc? o No o Yes

If Yes, give details below

_____

_____

4. Have you ever had an operation on your bowel?

o No o Yes

If Yes, give details below

_____

5. Please give details of any other operations that you have had

(including removal of tonsils/appendix etc.)

6. What medications (including laxatives) do you take regularly?

Drug name	Duration of use	Dose/amount	Times per day	Regular or when needed

7. Do any medical conditions run in the family?

o No o Yes

If Yes, give details below

TO BE COMPLETED BY WOMEN ONLY

1. Have you ever had a hysterectomy or other operation on your womb or vagina?

o No o Yes

If Yes, give details below

2. Childbirth History

Number of Deliveries: _____

For each delivery please tick appropriate box:

Delivery Number	Year	Normal Vaginal Delivery	Vaginal Delivery with Tear/Episiotomy	Suction	Forceps	Caesarean Section
1						
2						
3						
4						
5						
6						
7						
8						

Thank you for taking the time to complete this questionnaire.

---

2. Health survey questionnaire for healthy volunteers (SF-36)

Today's Date: _____

Name: Last: _____ First: _____

Date of Birth: _____

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

Please answer these questions by "check-marking" your choice. Please select only one choice for each item.

1. In general, would you say your health is:

1.Excellent 2.Verygood 3.Good 4.Fair 5.Poor

2. Compared to ONE YEAR AGO, how would you rate your health in general NOW?

Much better than one year ago

Somewhat better now than one year ago

About the same as one year ago

Somewhat worse now than one year ago

Much worse now than one year ago

3. The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

Activities	**1. Yes, Limited A Lot**	**2. Yes, Limited A Little**	**3. No, Not Limited At All**
a) **Vigorous activities,** such as running, lifting heavy objects, participating in strenuous sports?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all
b) **Moderate activities,** such as moving a table, pushing a vacuum cleaner, bowling, or playing golf?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all
c) Lifting or carrying groceries?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all
d) Climbing **several flights** of stairs?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all
e) Climbing **one** flight of stairs?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all
f) Bending, kneeing or stooping?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all
g) Walking **more than a mile**?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all
h) Walking **several** blocks?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all
i) Walking **one** block?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all
j) Bathing or dressing yourself?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all

4. During the past 4 weeks, have you had any of the following problems with your work or other regular activities as a result of your physical health?

	Yes	No
a) Cut down on the **amount of time** you spent on work or other activities?	1. yes	2. No
b) **Accomplished less** than you would like?	1. yes	2. No
c) Were limited in the **kind** of work or other activities?	1. yes	2. No
d) Had **difficulty** performing the work or other activities (for example it took extra effort)?	1. yes	2. No

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	Yes	No
a) Cut down on the **amount of time** you spent on work or other activities?	1. yes	2. No
b) **Accomplished less** than you would like?	1. yes	2. No
c) Didn't do work or other activities as **carefully** as usual?	1. yes	2. No

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?

1.Not at all 2.Slightly 3.Moderately 4.Quite abit 5.Extremely

7. How much bodily pain have you had during the past 4 weeks?

1. None 2. Very mild 3. Mild 4. Moderate 5. Severe 6. Very severe

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

1.Not at all 2.A little bit 3.Moderately 4.Quite abit 5.Extremely

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 week

	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time
a) Did you feel full of pep?	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time
b) Have you been a very nervous person?	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time
c) Have you felt so down in the dumps that nothing could cheer you up?	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time
d) Have you felt calm and peaceful?	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time
e) Did you have a lot of energy?	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time
f) Have you felt downhearted and blue?	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time
g) Do you feel worn out?	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time
h) Have you been a happy person?	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time
i) Did you feel tired?	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

All of the time

Most of the time.

Some of the time

A little of the time.

None of the time.

11. How TRUE or FALSE is each of the following statements for you?

	1. Definitely true	2. Mostly true	3. Don't know	4. Mostly false	5. Definitely false
a) I seem to get sick a little easier than other people?	1. Definitely true	2. Mostly true	3. Don't know	4. Mostly false	5. Definitely false
b) I am as healthy as anybody I know?	1. Definitely true	2. Mostly true	3. Don't know	4. Mostly false	5. Definitely false
c) I expect my health to get worse?	1. Definitely true	2. Mostly true	3. Don't know	4. Mostly false	5. Definitely false
d) My health is excellent?	1. Definitely true	2. Mostly true	3. Don't know	4. Mostly false	5. Definitely false

3. Bristol scoring system for stool form

By comparing your stool consistency to the chart below:

PLEASE ANSWER EACH QUESTION a – c

Type 1 	Separate hard lumps, like nuts (hard to pass)
Type 2 	Sausage-shaped but lumpy
Type 3 	Like a sausage but with cracks on its surface
Type 4 	Like a sausage or snake, smooth and soft
Type 5 	Soft blobs with clear-cut edges (passed easily)
Type 6 	Fluffy pieces with ragged edges, a mushy stool
Type 7 	Watery, no solid pieces ENTIRELY LIQUID

a. How often are your stools types 1 & 2

Never	O
Rarely (less than a quarter of the time)	O
Sometimes (a quarter to half of the time)	O
Usually (more than half of the time)	O
Always	O

b. How often are your stools types 3, 4 & 5

Never	O
Rarely (less than a quarter of the time)	O
Sometimes (a quarter to half of the time)	O
Usually (more than half of the time)	O
Always	O

c. How often are your stools types 6 & 7

Never	O
Rarely (less than a quarter of the time)	O
Sometimes (a quarter to half of the time)	O
Usually (more than half of the time)	O
Always	O

4. Screening questionnaire to recruit healthy volunteers provided during screening Visit

Subject Number:

☐☐☐

Date of Visit:

MM DD YYYY

☐☐ ☐☐ ☐☐☐☐

Date Informed Consent Signed:

MM DD YYYY

☐☐ ☐☐ ☐☐☐☐

Did the Subject Meet all Eligibility criteria? ☐1 Yes ☐0 No

Gender

☐1 Male ☐2 Female

Race

☐1 Caucasian

☐2 Black

☐3 Asian/Pacific Islander

☐4 Hispanic

☐5 American Native

☐98 Other (S*pecify*)_____

Date of birth:

MM DD YYYY

☐☐ ☐☐ ☐☐☐☐

Height:

(in inches)

☐☐.☐

Weight:

(in pounds)

☐☐☐.☐

Does the subject smoke cigarettes? ☐1 Yes ☐0 No

If Yes, how many cigarettes does the subject smoke each day?

☐1 Less than 1 pack ☐2 1-2 packs ☐3 3 or more packs

COMPLETE THIS SECTION *only* if Subject is a FEMALE

Urine Pregnancy Test –FEMALE

☐ 95 N/A (Post menopausal/surgery) ☐ 94 Not Done (If not done and the subject is of childbearing potential, *do not enrol*)	Result: ☐ 1 Positive* ☐ 0 Negative (*If Positive, *do not enrol*)

Subject Number:

☐☐☐

GENERAL MEDICAL HISTORY

(present or past, including surgical interventions)

SYSTEM	SYMPTOMS Yes, No, or Unknown *(Check one)*	If yes, specify
Head, ears, nose, throat	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Cardiovascular	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Peripheral vascular	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Respiratory	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Gastroesophageal reflux	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Ulcer(s)	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Irritable Bowel Syndrome	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Hepatobiliary	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Renal	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Genitourinary	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Endocrine-metabolic (Include diabetes)	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Hematologic lymphatic	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Musculoskeletal	☐ 1 Yes ☐ 0 No ☐ 97 Unk	

(Include arthritis)		
Dermatologic	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Neurologic	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Psychiatric	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Neoplasia	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Alcohol Use	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Drugs Use	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Allergies	☐ 1 Yes ☐ 0 No ☐ 97 Unk	
Other (*specify*)		

PHYSICAL EXAM		
SYSTEM	Was the Exam Normal? Yes, No, or Not Done *(Check one)*	If no, specify
General Appearance	☐ 1 Yes ☐ 0 No ☐ 94 ND	
Head, ears, nose, throat	☐ 1 Yes ☐ 0 No ☐ 94 ND	
Neck	☐ 1 Yes ☐ 0 No ☐ 94 ND	
Heart	☐ 1 Yes ☐ 0 No ☐ 94 ND	
Lungs	☐ 1 Yes ☐ 0 No ☐ 94 ND	
Abdomen	☐ 1 Yes ☐ 0 No ☐ 94 ND	
Lymph Nodes	☐ 1 Yes ☐ 0 No ☐ 94 ND	
Renal	☐ 1 Yes ☐ 0 No ☐ 94 ND	

Genitourinary	☐ 1 Yes ☐ 0 No ☐ 94 ND	
Extremities	☐ 1 Yes ☐ 0 No ☐ 94 ND	
Neurological	☐ 1 Yes ☐ 0 No ☐ 94 ND	
Skin	☐ 1 Yes ☐ 0 No ☐ 94 ND	
Musculoskeletal	☐ 1 Yes ☐ 0 No ☐ 94 ND	
Other (*specify*)		

Record any medications taken

Subject Number: ☐☐☐		
In the last 3 months, did you *often* have pain in your upper abdomen (above your belly button, or the pit of your stomach)?		

GASTROINTESTINAL DISEASE QUESTIONNAIRE FOR SCREENING HEALTHY VOLUNTEERS		
	YES	NO or rarely
1. In the last 3 months, did you *often* have discomfort centered in your upper abdomen (above your belly button, or the pit of your stomach)? If the answer is yes, which of the following describe your discomfort (check all that apply) Nausea Bloating (a sensation of upper abdominal swelling) Feeling full after eating very little None of the above		
2. In the last 3 months did you have frequent episodes of vomiting (on at least 3 separate days in each week)?		
3. In the last 3 months did you *often* have discomfort		

in your abdomen (below the belly button)? If the answer is yes, which of the following describe your discomfort (check all that apply) ☐ Abdominal fullness ☐ Bloating (a sensation of lower abdominal swelling) ☐ None of the above		

4. Have you had any of the following symptoms at least one-quarter of the time in the last 3 months ☐ Fewer than three bowel movements a week (0-2) ☐ More than three bowel movements a day (4 or more) ☐ Hard or lumpy stools ☐ Loose, mushy or watery stools ☐ Straining during a bowel movement ☐ Having to rush to the toilet to have a bowel movement ☐ Feeling of incomplete emptying after a bowel movement ☐ A sensation that the stool cannot be passed (i.e. blocked) when having a bowel movement ☐ A need to press on or around your bottom or vagina to remove stool in order to complete the bowel movement		
5. In the last 3 months did you often have pain in your abdomen (below the belly button) *(if you are female, this should not be related to your menstrual cycle or period)*		

Often means that the symptoms were present during at least 3 weeks (at least one day in each week) in the last 3 months.

If the subject is a healthy volunteer and answers YES to any of the above questions DO NOT ENROLL.

5. Pancolonic manometry catheters cleaning protocols as agreed with the infection control team at Barts Health Trust (for water-perfused catheter) and according to the recommendation from the manufacturer (for solid-state catheter) (UniTip: Unisensor AG, Attikon, Switzerland).

CLEANING PANCOLONIC MANOMETRY CATHETER

Solid-state catheter (20 ch)

Water-perfused catheter (16 ch)

Wash thoroughly by using tap water for at least 1 min as it has faecal material. Please note do not expose the connectors at the end of the catheter to any water or other cleaning agent.

Done by..... Date.....

Take out any thread from the tip of the catheter with extreme care and do not use any sharp instrument.

Done by..... Date.....

Wipe the catheter carefully with soft gauze or tissue (Alcohol-free)

Done by..... Date.....

Place the catheter in the cleansing solution (Deconex 36 intensive®) (code S-2) for NO MORE THAN 30 minutes.

Done by..... Date.....

Flush the lumen (s) of the catheter at least 3 times using the same cleansing solution with the use of 10 ml syringe at the beginning of the 30 minutes period. Do the same procedure before taking out the catheter from the Deconex solution. (Everything should be done within the 30 minutes period)

Done by..... Date.....

Wash the catheter again with clear slightly worm water and flush the lumen (s) with the clear water at least 3 times. Later on wipe it with clean gauze.

Done by..... Date.....

DO EXACTLY THE SAME STEPS (4, 5, 6) as described before, but with the use of sterilisation solution (Gigiased) (code S-1).

Done by..... Date.....

Lastly, flash the lumen with the air 2-3 times

Done by..... Date.....

Keep it to dry in a clean sheet

Done by..... Date.....

ALWAYS WEAR A MASK AND GLOVES AND A WHITE COAT AS THE STERILISATION SOLUTION CAN SPLASH WHEN FLUSH THROUGH THE CATHETER PORT